Temperature-Controlled Retinal Photocoagulation – A Step Toward Automated Laser Treatment

Stefan Koinzer, Kerstin Schlott, Lars Ptaszynski, Marco Beyer, Susanne Kleemann, Mark Saeger, Alexander Baade, Yoko Miura, Reginald Birngruber, Ralf Brinkmann, and Johann Roiber

PURPOSE. Retinal laser photocoagulation carries the risk of overtreatment due to effect variation of identically applied lesions. The degree of coagulation depends on the induced temperature increase and on exposure time. We introduce temperature controlled photocoagulation (TCP), which uses optoacoustics to determine individually exposure times necessary to create reproducible lesions.

METHODS. Optoacoustic temperature measurement relies on pressure waves that are excited in the retinal tissue by repetitive low-energy laser pulses. Signal amplitudes correlate with tissue temperature and are detected by a transducer in the laser contact lens. We used a continuous wave (CW) photocoagulator for treatment irradiation and superimposed probe laser pulses for simultaneous temperature measurement. Optoacoustic data of 1500 lesions (rabbit) were evaluated to develop an algorithm that controls exposure times automatically in TCP. Lesion diameters of 156 TCP lesions were compared to 156 non-controlled lesions. Histology was performed after 1 hour, and 1 and 4 weeks.

RESULTS. TCP resulted in exposure times from 4 to 800 ms depending on laser power chosen. Ophthalmoscopic and histologic lesion diameters were independent of power between 14 and 200 mW. TCP lesions barely were visible with a mean diameter equal to the treatment beam (130 μm). In contrast, standard lesion diameters increased linearly and statistically significantly with power. Histology confirmed sparing of the ganglion and nerve fiber layers in TCP.

CONCLUSIONS. TCP facilitates uniform retinal lesions over a wide power range. In a clinical setting, it should generate soft and reproducible lesions independently of local tissue variation and improve safety, particularly at short exposure times.

Retinal laser photocoagulation typically requires permanent power adaptation according to the ophthalmoscopic appearance achieved by previous exposures. Inter- and intraindividual changes in optical transmission, retinal pigmentation, and other factors contribute to variable retinal effects achieved despite identical exposure parameters. Despite spot-to-spot power control by the treating physician, significant overdosage of single spots in approximately one percent of treatment sessions is inevitable. To control the process of photocoagulation, realtime optoacoustic retinal temperature measurement has been introduced in transpupillary thermotherapy (TTT). More recently, the technique was developed further to allow temperature monitoring during retinal photocoagulation, where the exposure times, commonly 20–200 ms, are much shorter than in TTT. First clinical measurements already have shown typical temperatures between 55 and 90°C for barely visible to mild lesions. The method can be used further to cease irradiation automatically when a certain tissue temperature has been achieved for a certain time. Since the spatiotemporal retinal temperature profile correlates to the tissue damage extent, the automatic laser-stop function allows the application of defined reproducible coagulations and improves the safety of photocoagulation, particularly for exposure times below 20 ms. Conventional post-exposure laser lesion control is replaced by temperature monitoring during photocoagulation, allowing prospective, non-invasive spot-individual automatic dosage control.

METHODS

Experimental Setup for Temperature Controlled Photocoagulation (TCP)

Optoacoustic signal generation and temperature measurements were achieved using a modified method as described in detail by Kandulla et al. In short, the beam of a Q-switched Nd:YLF laser (λpulse = 523 nm, τpulse = 75 ns, f = 1 kHz, “probe laser”) was coupled collinearly into the beam of a continuous wave (CW) Nd:YAG photocoagulator (λcw = 532 nm, tcw = 4 – 800 ms, Pcw = 10 – 400 mW, “treatment laser”) to apply repetitive nanosecond laser pulses onto the heated fundus tissue during photocoagulation (Fig. 1). Each laser pulse causes a short and small temperature rise with a subsequent thermoelastic expansion of the tissue, generating a temperature-dependent optoacoustic pressure wave that is propagating through the whole eye. These pressure waves are detected at the cornea by an ultrasonic transducer that is coupled to the eye by a water-based coupling gel.
embedded in a laser contact lens (modified Mainster focal grid, OMRAS, manufactured by Medical Laser Center Lübeck GmbH, Lübeck, Germany). The piezolectric transducer signals were amplified and then digitized by a fast computer oscilloscope card (CompuScope 8347, Gage Applied Technologies, Lockport, IL). Data processing and analyzing of the optoacoustic signal development were executed by a real-time LabVIEW routine. Measurements were performed at a probe laser repetition rate of 1 kHz, giving a series of 200 measurements during an exposure of 200 ms duration. Optoacoustic signal values were normalized to body temperature by applying 20 probe pulses before laser treatment for each spot, allowing the calculation of absolute temperatures as described previously.\(^3\)\(^4\)\(^5\)\(^7\) Since tissue denaturation depends mainly on maximum tissue temperature, and optoacoustic signals correlate to the tissue temperature, optoacoustics allow monitoring of tissue damage extent. Based on optoacoustic data from approximately 1500 laser photocoagulation lesions, we developed an algorithm that calculates exposure times necessary to achieve reproducible fundus lesions (Schlott, J Biomed Optics. 2012, accepted for publication). We defined the desired fundus lesions to be mild and as large as the pilot spot, allowing prediction of the ophthalmoscopically visible damage extent. The algorithm calculating appropriate exposure times was integrated into a real-time LabVIEW routine that controls laser exposure times automatically.

**Evaluation of the End Temperature**

The highest temperature during irradiation occurs in the center of the spot and at the end of the irradiation time. To compensate for noise and interferences of the measured temperature curve, an exponential fit function was applied. The fit function was found by solving the heat equation semi-analytically for a rabbit fundus tissue model.\(^7\) The end temperature then was obtained from this fit.

**Animal Experiments**

We used 13 chinchilla grey rabbits for laser photocoagulation, 2 of which were euthanized for histological workup. They were treated under general anesthesia (ketamine 10\% 0.5–0.7 mL/kg, xylazine 2\% 0.2–0.25 mL/kg applied intramuscularly), and body temperature was monitored by rectal measurements. Pupils were dilated by phenylephrine (5\%) and tropicamide (5\%) eyedrops, and local anesthesia was administered with tetracaine (5\%) eyedrops. The modified laser contact lens was fitted onto the eye with methylcellulose gel (2\%) and fixed mechanically in its position. Fundus color images and fluorescence angiograms (FLAs, 0.5–1.0 mL fluorescein 10\% intravenously) were recorded one hour after the photocoagulation session.

All rabbits were maintained in animal units at the University Medical Center of Schleswig-Holstein, and all animal experiments were performed according to the German law for protection of animals (approved by the Ministry of Agriculture, the Environment and Rural Areas of Schleswig-Holstein, Kiel, Germany) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Evaluation of TCP**

TCP was tested on rabbit eyes. Individual spot mapping was facilitated by marker lesions that had been applied to the fundus several months before the experiments. To compare standard laser lesions with an exposure time of 200 ms to TCP lesions, six lesion columns were applied below the visual streak at nine power steps ranging from 14 mW (sub-visible) to 200 mW (strong suprathreshold or retinal rupture). Matched TCP lesions with the same power setting were applied next to standard lesions. To exclude any systematic local influence on the effect size, such as retinal thickness variations, or image distortion by the degree of optical eccentricity, we applied the columns with alternately increasing or decreasing power downward from the visual streak. An equal number of columns was applied right and left below the visual streak at nine power steps ranging from 14 mW (sub-visible) to 200 mW (strong suprathreshold or retinal rupture). Matched TCP lesions with the same power setting were applied next to standard lesions. To exclude any systematic local influence on the effect size, such as retinal thickness variations, or image distortion by the degree of optical eccentricity, we applied the columns with alternately increasing or decreasing power downward from the visual streak. An equal number of columns was applied right and left below the visual streak. The diameter of the treatment laser spot on the rabbit fundus was 130 \(\mu\)m. The same treatment was repeated at 3 different time points, producing lesions that were 1 hour, and 1 and 4 weeks old for histological investigation. A total of 156 standard and 156 TCP lesions were applied. Lesion diameters, FLA dye leakage and histological specimens were evaluated.

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**Figure 1.** Schematic illustration of the experimental setup. The beams of two laser systems, a Q-switched Nd:YLF probe laser (\(\lambda = 523 \text{ nm}\)) working at a repetition rate of 1 kHz and a CW Nd:YAG treatment laser (\(\lambda_{\text{CW}} = 532 \text{ nm}\)), are coupled into the same fiber. This allows the application of pulsed and CW irradiation simultaneously via a standard laser slit lamp. The probe pulses induce temperature-dependent acoustic pressure waves from the fundus. These can be detected on the ocular surface by an ultrasonic ring transducer, which is integrated into the laser contact lens. The optoacoustic signals are amplified and digitized by a fast computer oscilloscope card. A real-time LabVIEW routine analyzes the signal development (the temperature profile, respectively) during photocoagulation and limits CW exposure via feedback control.
Assessment of Lesion Size

Lesion diameters were assessed on digital color fundus images taken 1 hour after the end of the treatment. To measure the size of a lesion, it was outlined manually by a circle in image editing software (Gimp Ver. 2.6.8, available at http://www.gimp.org). All marked circles' pixel sizes were determined semi-automatically by ImageJ software (Ver 1.42q, National Institutes of Health, Bethesda, MD, available at http://rsbweb.nih.gov/ij/), and the pixel and real diameters calculated. A scaling factor for the pixel-to-μm calculation had been obtained from marker lesions with a defined center-to-center distance in preliminary experiments (the scaling factor was found to be 9.5 ± 0.7 μm per pixel using a Zeiss VISUCAM [Carl Zeiss Meditec AG, Jena, Germany]). Edematous halos were excluded from the spot size measurements.

The size of study lesions was assessed manually by three independent observers. Two observers performed one measurement per spot and the third observer performed three measurements per spot. Five measurements total per spot were performed, out of range-values were excluded and mean values were considered to be the spot size.

Histological Analysis

For histological analysis, enucleated eyes were fixated overnight in Margo’s solution (1% buffered formaldehyde and 1.25% glutaraldehyde). The area of interest was cut out of a retina-choroid-sclera preparation, marked with color, embedded in paraffin, cut in 5 μm sections at 50 μm steps, and stained with hematoxylin and eosin. Marker lesions, color marks, the optic nerve, and the spatial relation of laser lesions were used to recognize laser spots within the specimens. We analyzed laser spots qualitatively and by largest diameter measurements.

Statistical Analysis

Statistical analysis of the proportions of subthreshold (invisible) lesions and of linear regression was performed by the statistical software R, version 2.10.1. Numbers of subthreshold lesions in both groups of laser lesions, standard versus TCP, were compared by Fisher’s exact test. The correlation of laser power and spot diameter was analyzed in a simple linear regression. The 95% confidence intervals (CI) for prediction and

### Table: Assessment of Lesion Size

<table>
<thead>
<tr>
<th>POWER [mW]</th>
<th>standard (200 ms) lesions</th>
<th>TCP lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>382 μm 108 °C</td>
<td>93 μm 5 ms 74 °C</td>
</tr>
<tr>
<td>150</td>
<td>402 μm 83 °C</td>
<td>118 μm 7 ms 70 °C</td>
</tr>
<tr>
<td>125</td>
<td>316 μm 73 °C</td>
<td>143 μm 6 ms 70 °C</td>
</tr>
<tr>
<td>100</td>
<td>300 μm sub-visible 66 °C</td>
<td>79 μm 6 ms 65 °C</td>
</tr>
<tr>
<td>75</td>
<td>266 μm 57 °C</td>
<td>20 μm 9 ms 57 °C</td>
</tr>
<tr>
<td>50</td>
<td>211 μm 60 °C</td>
<td>sub-visible 13 ms 66 °C</td>
</tr>
<tr>
<td>40</td>
<td>155 μm 58 °C</td>
<td>156 μm 86 ms 58 °C</td>
</tr>
<tr>
<td>25</td>
<td>invisible 50 °C</td>
<td>153 μm 800 ms 54 °C</td>
</tr>
</tbody>
</table>

**Figure 2.** Comparison of standard 200 ms lesions (left) and TCP lesions (right) that were applied with identical laser power in each line (λ = 532 nm, effective spot size 130 μm), increasing from bottom to top (subthreshold to strong suprathreshold) as indicated on the left. Color fundus images and corresponding FLAs are shown. Lesion diameters and end temperatures are indicated next to each lesion (n.a., not available). For TCP-lesions, individually applied exposure times also are indicated. All temperature values represent end temperatures during laser irradiation. Constant exposure times of 200 ms in the left column show increasing clinical effects, diameters, and temperatures with increasing power. TCP lesions in the right column are homogeneous and very soft, sometimes only visible in FLA (~20% of lesions). Low peak temperatures require longer exposure times to produce the same ophthalmoscopic effect. At very short exposure times (<10 ms), accuracy of temperature determination and of exposure calculation is decreased, causing a tendency toward stronger lesions, but reliably preventing rupture or bleeding.

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RESULTS

Qualitative Evaluation of Standard and TCP Lesions

Figure 2 displays two representative columns of laser lesions with their corresponding FLAs. The left column shows 200 ms standard exposures and the right column shows TCP lesions. Laser power increases from bottom (25 mW) to top (175 mW). End temperatures also are displayed, which represent spatial and temporal peak values within the irradiated tissue volume (Fig. 3). Figure 3 shows examples of temperature profiles obtained from some spots in Figure 2.

Standard exposures cause increasing clinical effects from bottom (invisible) to top (rupture) as power and temperature increase, respectively. TCP produces almost homogeneous and soft effects with gentle to moderate leakage in FLA. Some TCP lesions are visible only in FLA. At very high power, TCP resulted in short exposure times below 10 ms with a tendency toward stronger lesions, but no rupture or bleeding occurred. A slight change in appearance of TCP lesions can be observed from rather dark discoloration with a bright ring at low power, and long exposure times to soft blanching with surrounding dark discoloration at higher power and short exposure times. Since dark discoloration represents pigment shedding from RPE cells, it was included in the damage zone measurements, especially in soft lesions.

Some representative temperature and optoacoustic data examples are presented below, and in Figures 2 and 3. Further details on temperature assessment and statistics have been published previously.5,4,7

Standard lesions (200 ms exposure time) showed increasing end temperatures with increasing power, starting at around 50°C (25 mW, invisible) and increasing from 60°C (slightly visible) beyond 108°C (100 mW, chalk white, temperature not determinable for 125–175 mW lesions). Temperature profiles show increasing slopes and increasing maxima as power increases (Fig. 3).

With TCP, the lesion applied with 25 mW reached 54°C at the exposure time limit of 800 ms and was slightly visible. As power and temperature increased to 40 mW/58°C and 50 mW/66°C, exposure times were reduced by TCP to 86 and 13 ms, respectively. At increasing power, many times leading to TCP laser shut off at very short times (<10 ms), the accuracy of temperature determination decreases significantly. Due to the sensor data being of limited validity, the shut-off algorithm has a tendency to overheat (Fig. 4). Despite that limitation, lesion diameters remained close to the irradiated diameter as intended. At very high power (125–175 mW), TCP temperatures reached at most 74°C, which is far below peak temperatures of control lesions, which rose above 108°C.

Complications and Clinical Visibility

One bleeding occurred among 156 standard 200 ms lesions. The TCP algorithm failed on one of 156 lesions due to optoacoustic signal interferences, resulting in maximum irradiation time of 800 ms as set by the examiner, causing a strong lesion with retinal rupture. With this exception, neither bleedings nor ruptures occurred among the TCP lesions. A few laser lesions were applied beyond the optical field of the fundus camera and were not eligible for graphic evaluation, resulting in 149 standard and 143 TCP lesions that could be evaluated.

Figure 4 displays the proportions of ophthalmoscopically visible standard and TCP lesions according to laser power. Considering the overall proportion of ophthalmoscopically invisible lesions among 145 TCP versus 149 standard lesions, there was no statistically significant difference as assessed by Fisher’s exact test. Looking at the subgroup in the medium power range (31–85 mW), TCP worked at its best and produced 80% ophthalmoscopically visible lesions. In the same power range, standard lesions were visible in 90–100%. If power was chosen beyond 85 mW, nearly all control lesions were invisible, but produced increasing retinal coagulation, while TCP, due to a tendency to overheat at short exposure times, produced 90–100% visibility without increasing coagulation effects. At powers below 31 mW, visibility rates of standard lesions were distributed randomly from 0–65%. TCP could counteract the threshold variability in part, producing 30% (13–19 mW) and 60% (20–30 mW) visible lesions. In cases where the coagulation laser did not produce enough heat to reach the threshold temperature, TCP was not effective anymore.

Qualitative Histological Evaluation

Figure 5 is a histologic composite of standard (200 ms) versus TCP lesions at increasing laser power and at different time points. The threshold power of clinical visibility within 1 hour was around 50 mW for 200 ms exposures.

Figure 5a shows specimens taken 1 hour after treatment. A 133 μm scale indicates the irradiation beam diameter. Black bars underneath each picture indicate the horizontal lesion size at the RPE/photoreceptor outer segment (POS) interface. Vertical damage extensions are indicated by asterisks.

The radial lesion size increases with power for 200 ms photocoagulation, but remains roughly constant in TCP lesions. At 22 mW power, which is likely to be ophthalmoscopically invisible (Fig. 4), the damage zones of standard (200 ms) and TCP (96 ms) lesions are comparable. At 44 mW power, which was about the threshold of ophthalmoscopic visibility, the standard lesion displays extensive coagulation of POS and pyknosis of photoreceptor nuclei, which are accompanied by pyknotic changes in the inner nuclear layer (INL) and mild
edema of the inner retinal layers. These axial changes increase as power increases. At 111 mW, the ganglion layer (GL) is coagulated completely. In TCP lesions, in contrast, pyknotic changes of the INL, if present, are very mild, and the innermost retinal layers remain completely unaffected.

Figure 5b shows specimens taken one week and one month after treatment. The 22 mW lesions were invisible histologically at these time points and are not shown. After one week, a healing reaction shows degradation of necrotic debris and early fibrosis of the affected retinal layers, including some invasion of the scar by big, round pigmented cells. In the 87 mW TCP lesion, histologic changes are minimal, indicating that the lesion was either sub-threshold or already has healed. At the 87 and 115 mW standard lesions, all retinal layers are affected by the tissue reaction, while in the corresponding TCP lesions, the inner retinal layers are unaltered.

After 1 month, necrotic photoreceptors are replaced by fibrotic tissue, and there is vacuolization at the RPE/POS interface in both lesion types. The INL appears largely intact and the innermost layers are unaltered completely in TCP lesions, while in standard lesions a power-dependent scarring is obvious, which is maximal for 124 mW and shows fibrosis of all retinal layers. Histologically, in TCP lesions no obvious correlation of histologic damage and laser power is found.

Standard lesions display involvement at least of the INL as soon as they become clinically visible (evaluated after 1–3 hours).

**Statistical Evaluation of Ophthalmoscopic Lesion Size (Fig. 6)**

Lesions applied at power below 50 mW were excluded from the regression analyses, because they were not reliably visible in ophthalmoscopy (Fig. 4, left). The size (diameter) and whitening of standard lesions increase with power (Fig. 6, left chart). Their almost linear relationship of power and lesion diameter above 50 mW is described best by the linear equation:

\[ y = 187 + 1.19x \]

where \( y \) is the fundus lesion size (\( \mu \)m) and \( x \) is the laser power (mW), with \( R^2 = 0.69, P < 0.001 \) and an average 95% CI width for prediction \( d = 135 \mu \)m (Fig. 6, left chart). Ideal lesions (130 \( \mu \)m) are not achievable with standard (200 ms) coagulations.

While the retinal image size of the treatment laser beam is 130 \( \mu \)m, the linear regression of lesion size to power in TCP lesions is described best by the linear equation:

\[ y = 134 - 0.039x \]
Representative histological results after standard 200 ms photocoagulation and TCP. Exposure times for TCP lesions are indicated. The threshold of ophthalmoscopic visibility (fundus image, 1–3 hours post) was around 40–50 mW, irradiation diameter was 130 µm for all lesions (red scale bar). Laser powers applied are indicated on the right beneath each pair of lesions. (a) Histological findings of lesions one hour post treatment. Asterisks indicate the axial damage extent. Black bars underneath indicate the diameter of histological damage at the interface of POS/RPE. It increases with power for standard lesions, but remains almost constant for TCP lesions. TCP damages are slightly bigger than an ophthalmoscopically invisible standard lesion (22 mW). Retinal layers as marked in left upper image: CHO, choroid; RPE, retinal pigment epithelium; POS, photoreceptor outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GL, ganglion layer.
with $R^2 = 0.0030$, $P = 0.65$ and $d = 117 \mu m$. The y axis intercept of $134 \mu m$ indicates the almost ideal lesion diameters, while the low slope of $-0.039$ shows the homogeneity of lesion diameters, and the low $R^2$ and high $P$ value depict the independency of lesion diameters from laser power. The CI of $117 \mu m$ is slightly lower in TCP, indicating somewhat improved reproducibility of lesions diameters compared to standard 200 ms lesions ($d = 135 \mu m$).

**DISCUSSION**

The advantages of a real-time objective laser exposure control were recognized for photocoagulation already in the 1970s.\textsuperscript{9–11} The technical implementation failed at that time, when exposure control was attempted by real-time reflectometric measurement of retinal whitening, leaving the task undone. Our study introduces TCP, a functional laser exposure control that relies on real-time optoacoustic temperature feedback.
during photocoagulation. TCP lesion diameters were independent of laser power ($P = 0.65$, $R^2 = 0.0030$), while standard photocoagulation lesions grew highly significant ($P < 0.001$) with increasing power in a linear fashion ($R^2 = 0.69$).

**Limitations of Conventional Laser Power Control**

During laser photocoagulation, light energy is absorbed in the RPE layer and causes local heating that diffuses into adjacent tissues, leading to their denaturation if a threshold temperature is exceeded long enough. The tissue denaturation, necrosis, and edema cause a loss of transparency due to increased light scattering, giving the retina a white appearance and changing the tissue’s response to laser irradiation.\(^1\) Disintegration of RPE cells results in cell rupture and pigment shedding, which appears homogeneously dark in areas where the overlying retina has remained transparent. Measuring the diameter of retinal discoloration has been established as a practicable measurement for the determination of tissue damage extent. However, it has limitations as retinal damage already is extensive when blanching first occurs.\(^6\)\(^,\)\(^1\)\(^2\)\(^,\)\(^1\)\(^3\) Moreover, blanching increases up to one day after photocoagulation. Its clinical use is of limited accuracy and will underestimate the histologic effect especially in soft lesions.

The biologic and therapeutic effect of retinal laser irradiation in the eye depends on the laser parameters of wavelength, beam diameter, exposure time, and power. It also depends on biological variables, like optical transmission, pigmentation variation, and differences in retinal anatomy.\(^1\)\(^3\)\(^,\)\(^1\)\(^4\) Even though the mean diameter of supra-threshold lesions correlates with laser power in a linear fashion, as it also has been shown by Jain et al.,\(^1\)\(^5\) this correlation does not allow reliable prediction of an individual lesion at all (95% CI width = 135 \( \mu \)m).

**Retinal Temperature Measurement**

The spatiotemporal temperature profile determines the morphological and biological alterations.\(^6\)\(^,\)\(^7\)\(^,\)\(^1\)\(^6\) Hence, retinal temperature measurement will allow to monitor photocoagulation in real-time and overcome the above mentioned limitations of conventional dosimetry. In early in vitro attempts to measure retinal temperatures during laser heating, tissue manipulation by thermal probes and probe displacement were shortcomings.\(^1\)\(^7\) A newer approach performing high-speed thermal imaging is not feasible simultaneously to photocoagulation due to strong absorption of infrared light within the eye.\(^1\)\(^8\) These limitations can be overcome by the optoacoustic method, which allows determination of in vivo tissue temperature changes every millisecond (at 1 kHz repetition rate\(^3\)\(^,\)\(^5\)). Probe laser irradiation and treatment laser irradiation are absorbed at identical sites, granting temperature assessment exactly at the targeted tissue structure. It is limited, however, to constant tissue properties. The tissue properties, particularly the light scattering and absorption characteristics, change when denaturation begins. Thus, when retinal blanching occurs, the temperature calibration data acquired before the photocoagulation become invalid, limiting the further temperature determination of strong supra-threshold lesions (Fig. 2). Consequently, optoacoustic temperature determination works well to evaluate laser lesions around denaturation threshold, but predictive temperature calculations will be necessary for a significant proportion of strong coagulations.

**Accuracy of TCP**

Since laser control in TCP relies on prospective analysis of optoacoustic data firsthand, TCP still will work with good functionality in many lesions where end temperature calculation is impossible. In our study, TCP was investigated to produce lesions that have a reproducible size just as large as the treatment beam diameter, producing about 20% ophthalmoscopically invisible lesions. However, adaptation of the method to other ophthalmoscopic lesion characteristics, for example soft or moderate blanching, or to any OCT characteristic, also will be possible.

Using TCP, it is possible to produce fundus lesions of a nearly constant mean diameter over a wide power range (Fig. 6). Aiming at very gentle lesions, TCP produced more sub-threshold lesions (20%) than standard treatment (0–10%) in the ideal power window of 31–85 mW. At higher power, sub-threshold lesions rarely occurred in TCP due to slight overdosage as discussed above. At power below 31 mW, fewer TCP than standard lesions were sub-threshold due to extended exposure up to 800 ms (Fig. 4).

TCP lesions achieved the proposed mean size clinically, but showed significant scattering ($d = 117 \mu m$) almost like standard lesions ($d = 135 \mu m$). The histological extent of TCP lesions showed some variability as well, ranging from subthreshold (no visible effect) to destruction of the outer retinal layers, sometimes including even parts of the INL. However, the GL and nerve fiber layer always was spared. This is crucial for photocoagulation safety, since coagulation of the GL and nerve fiber layer will cause collateral damage outside the treated area. As histology revealed, standard lesions applied with supra-threshold power (>50 mW) show coagulation of the GL in a significant proportion (Fig. 6). Consequently, the method presented in our study has the potential to improve reliability and safety of photocoagulation, avoiding rupture, bleeding, and GL coagulation especially for short exposure times (<20 ms).

Morphometric lesion characterization seemed appropriate to adjust the TCP algorithm and demonstrate functionality of the method. In a further step, more specific functional assessment, such as electroretinography (ERG) or microperimetry, and more specific biological assays on cell survival, cytokine expression, inflammation, and others will be helpful to gain more detailed understanding of TCP.

**Challenges in Transferring TCP to Human Patients**

TCP has been developed and tested in a Chinchilla grey rabbit model, which grants ideal conditions for photocoagulation. In this clear media and a particularly homogeneous fundus pigmentation, and are free of any retinal pathology. Moreover, the experimental setup with general anesthesia and mechanical fixation of the laser contact lens largely excluded any motion artifacts. These will be introduced by manual fixation of the lens. Movements may produce interfering acoustic signals by modulation of the contact lens pressure onto the cornea. However, animal experiments with a handheld contact lens still allowed sufficient optoacoustic data quality and TCP treatment.

Anatomical properties of human eyes deviate significantly from those of a rabbit. The retina and its sublayers have different thicknesses, with greater variation at different anatomical locations, and the pigmentation is more inhomogeneous. The intraretinal vasculature may influence the impact of photocoagulation. Significant pathologic alterations of human eyes undergoing photocoagulation add up to the uncertainties that must be clarified, such as media opacities, retinal ischemic condition, edemas, exudates, and others. It would be useful to conduct an OCT-temperature study on humans to establish the correlation of photocoagulation temperatures and resulting retinal effects, investigating possible influences of anatomical location and retinal pathologies.
However, successful application in rabbits has been the justification for testing innovative laser technology in humans before, such as selective retinal therapy (SRT).\textsuperscript{19,28,29}

Short Exposure Times in Photocoagulation

Lesions applied with shorter exposure times have a more homogeneous spatial temperature distribution over the lesion volume,\textsuperscript{22} making them less painful for the patient\textsuperscript{23} and reducing axial damage. On the other hand, the therapeutic window, which is defined to be the ratio of rupture threshold power to soft blanching threshold power, decreases for short exposure times.\textsuperscript{15,24} The shortest times accepted to be safe in the literature are between 10 and 50 ms.\textsuperscript{22,25}

The shortest exposure times applied in this TCP study were 4 ms. The accuracy of the method is reduced for such short times because of technical limitations of our experimental setup. Laser exposure termination will not be triggered quick enough in these cases, so there is a systematic error of overtreatment. This fact was confirmed experimentally by brighter retinal whitening (Fig. 2) and a lower percentage of invisible lesions (Fig. 4). Despite this limitation, even the shortest exposed TCP lesions do not cause bleeding or rupture, and peak temperatures remain well below those of high power standard lesions (>107°C).

Implications for Pattern Scanning Photocoagulators

Short exposure times are a prerequisite for laser pattern application, because the entire pattern must be applied faster than the patient’s eye will move. The maximum treatment time that generally is accepted per pattern is around 500 ms.\textsuperscript{26} Spot exposure times used commonly by pattern systems, like Zeiss VISULAS VITE (Carl Zeiss Meditec AG, Jena, Germany) or PASCAL (Topcon Europe Medical B.V., Capelle a/d Ussel, Netherlands), are 10–50 ms per spot.\textsuperscript{23,25–27} Significant overtreatment is more likely to occur at these short exposure times,\textsuperscript{22} although most published data show low rates of overtreatment.\textsuperscript{1,27} TCP, which facilitates safe exposure even at times as short as 4 ms, would contribute to increased safety and speed of pattern photocoagulation laser systems.

Micropulse Laser Treatment

The introduction of micropulse laser therapy was a cornerstone to facilitate mild photocoagulation, since micropulse treatment allows thermomechanical RPE cell disintegration without thermal collateral damage to the adjacent photoreceptors.\textsuperscript{19,20,28,29} The selectivity of the procedure, however, depends on the correct dosage. If the energy exceeds a threshold, partial or full thickness coagulation of the retina will occur with micropulse laser as it does with continuous wave laser. In SRT, dosage is controlled by titration of the threshold of ophthalmoscopic visibility outside the macula, and by optoacoustic-assisted energy reduction for the treatment spots. The treatment effect is confirmed routinely by fluorescence angiography shortly after the therapy. In SRT, the laser induces melanosome evaporation in the RPE. Every cavitation bubble generates a pressure wave, which can be recorded as acoustic signal by a transducer comparable to the one used in our study. These thermomechanical pressure waves are much stronger than the optoacoustic signal that we use to determine the retinal temperature. Therefore, melanosome evaporation is an undesired effect in TCP, making temperature control impossible, while it represents the therapeutic effect in SRT and is to be detected.

Temperature-Controlled Retinal Photocoagulation

Advantages of Minimally Invasive TCP Treatment

Since to our knowledge human retinal photocoagulation temperatures have never been systematically measured,\textsuperscript{4} further research is needed to establish the exact correlation of retinal temperature profiles, and axial and radial lesion extent. Reproducible and soft laser lesions may reduce pain, because limiting the axial damage also will reduce choroidal damage, sparing nociceptive nerve fibers. Additionally, it will give better functional results by avoiding GL damage, granting a sufficient tissue effect yet. A method to apply lesions with an adjustable and predictable extent will open new clinical research perspectives in re-defining the minimal effective dose of laser coagulation, which obviously is overestimated by Early Treatment Diabetic Retinopathy Study (ETDRS) standards.\textsuperscript{21,50} There is evidence that even sub-threshold treatment is effective in a variety of diseases, including diabetic macular edema, diabetic retinopathy, central serous chorioretinopathy, or subretinal fluid accumulation after retinal detachment surgery.\textsuperscript{19–21,20,29} but most reports are of the pilot type and need further statistical support.

Conclusions

We present TCP, a method that allows real-time, spot-by-spot laser exposure dosage by optoacoustic temperature measurement feedback and securely prevents overtreatment with sparing of the ganglion layer in rabbits. In contrast, conventional dosage, which relies on ophthalmoscopic post-exposure lesion evaluation, often produces extensive and unpredictable retinal effects, many times unintentionally coagulating the inner retinal layers. When TCP is transferred to clinical use, it will grant homogenous and well-defined retinal lesions.

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References


