Automatic irradiation control by an optical feedback technique for selective retina treatment (SRT) in a rabbit model

Eric Seifert*[a], Young-Jung Roh[b], Andreas Fritz[a], Young Gun Park[b], Seungbum Kang[b], Dirk Theisen-Kunde[a], Ralf Brinkmann[a]
[a]Medizinisches Laserzentrum Lübeck GmbH, Peter-Monik-Weg,4, 23562, Lübeck, Germany
[b]Department of Ophthalmology, Yeouido St. Mary's Hospital, College of Medicine, The Catholic University of Korea, No. 62 Yeouido-dong, Yeongdeungpo-gu, Seoul 150-713, Republic of Korea

ABSTRACT

Selective Retina Therapy (SRT) targets the Retinal Pigment Epithelium (RPE) without effecting neighboring layers as the photoreceptors or the choroid. SRT related RPE defects are ophthalmoscopically invisible. Owing to this invisibility and the variation of the threshold radiant exposure for RPE damage the treating physician does not know whether the treatment was successful or not. Thus measurement techniques enabling a correct dosing are a demanded element in SRT devices. The acquired signal can be used for monitoring or automatic irradiation control. Existing monitoring techniques are based on the detection of micro-bubbles. These bubbles are the origin of RPE cell damage for pulse durations in the ns and µs time regime 5µs. The detection can be performed by optical or acoustical approaches. Monitoring based on an acoustical approach has already been used to study the beneficial effects of SRT on diabetic macula edema and central serous retinopathy. We have developed a first real time feedback technique able to detect micro-bubble induced characteristics in the backscattered laser light fast enough to cease the laser irradiation within a burst. Therefore the laser energy within a burst of at most 30 pulses is increased linearly with every pulse. The laser irradiation is ceased as soon as micro-bubbles are detected. With this automatic approach it was possible to observe invisible lesions, an intact photoreceptor layer and a reconstruction of the RPE within one week.

Keywords: RPE, selectivity, dosimetry, feedback, SRT, laser, realtime, microsecond

1. PURPOSE

Selective retina therapy (SRT) is a laser method which targets the retinal pigment epithelium (RPE) with repetitive microsecond laser pulses, while causing no damage to the photoreceptors as well as the choroid [1]. Micro-bubbles arising at the melanosomes within the RPE cells are the origin of selective RPE cell death [2]. This makes SRT to a potential treatment tool for several retinal diseases. Beneficial effects on Central Serous Retinopathy (CSR) [3] and Diabetic Macula Edema (DME) [4] have already been shown. The pigmentation variation of the RPE [5] and the choroid [6] makes appropriate laser dosage non-predictable and the invisibility of selective lesions makes an ophthalmoscopic detection not possible. Thus techniques enabling correct dosing are a demanded element in SRT devices. Therefore the detection of microbubbles as the indication of RPE cell damage is appropriate. This can be done by optical or opto acoustical techniques. Opto-acoustic techniques have been implemented successfully but usually require a burst of pulses of the same pulse energy to detect signals caused by the micro-bubbles [7]. Optical techniques making use of the higher light reflectivity during micro bubble lifetime have also been investigated. In this work we present an optical technique to detect micro bubble formation based on the evaluation of backscattered light designed to detect micro-bubbles by analyzing each single laser pulse. For an automatic dosage control the system increases the pulse energy stepwise linearly with every pulse and ceases the laser irradiation automatically as soon as a predefined threshold-value is reached. We evaluated the safety, selectivity and healing response of the retinal lesions on rabbits by using the automatic irradiation control.

*a seifert@mll.uni-luebeck.de; 0049 451 500 6517; fax 0049 451 500 6546;

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2. METHODS

2.1 Animals and Tests

The chinchilla bastard rabbits were anesthetized with zoletil® (Vibrac, Carros, France; 0.2 mg/kg of body weight) andxylazine hydrochloride (5 mg/kg of bodyweight). The animals in this study were treated in compliance with the ARVO Statement for the use of animals in ophthalmic and vision research.
Two points of interest have been defined for RPE damage evaluation: lesion visibility in the fundus and visibility on fluorescein angiography images as an indicator for a breakdown of the blood retina barrier (RPE). For angiography 0.5 ml 10 % fluorescein sodium has been injected into the ear vein. Furthermore optical coherence tomography (Carl Zeiss, Stratus III) scans were taken to observe the neural retina. The diagnostic procedures have been repeated at 3 time points from 1 hour to 3 weeks.
The animals were sacrificed with an overdose of KCL, and the eyes were studied 3 weeks after treatment for histologic analysis. The tissue was prepared by immersion fixation in 4% glutaraldehyde, with removal of the anterior parts within 15 minutes. The eyes were fixed for 12 to 24 hours in cool condition, trimmed to block size after fixation, dehydrated in ethanol, and embedded in five micron sections were stained with hematoxyl and eosin (H & E).

2.2 Treatment Setup

Ten eyes of chinchilla bastard rabbits were treated with a SRT device based on a Q-switched Nd-YLF laser (wavelength: 527 nm, pulse duration: 1.7 µs, repetition rate: 100 Hz). The number of pulses in a burst was limited to a maximum of 30. The pulse energy has been increased with every pulse within a burst linearly by an acusto-optic modulator (AOM). The maximum energy was set between 85 µJ to 100 µJ. The applied pulses are acquired by a photodiode to calculate the applied pulse energy with a calibrated PC-system. The optical fiber creates a spot with a speckle factor F = 3.6 calculated by dividing the maximal radiant exposure $H_{\max}$ by the mean radiant exposure H_{mean} (measured with the Beam analyzer BC106-Vis of Thorlabs):

$$F = \frac{H_{\max}}{H_{mean}} \tag{1}$$

An optical system attached to a slit lamp is used to create an illuminated area of the desired spot size of 120 µm on the retina. To recalculate the maximum radiant exposure of the treated spot the ratio of the measured pulse energy E_{Pulse} and the spot size A_{Spot} (the mean radiant exposure) needs to be multiplied with the speckle factor.

$$H_{\max} = F \cdot \frac{E_{Pulse}}{A_{Spot}} \tag{2}$$

The backscattered light is separated and guided into a fiber leading to a photodiode. The photodiodes voltage is sampled with 100 MS/s. The digitized signals are analyzed by an algorithm which quantifies the micro-bubble characteristics for each pulse within 2 ms. The more and larger micro-bubbles are induced the higher the value. Parallel to the optical feedback method an acoustic method has been used to record micro-bubble induced ultrasonic transients. The signals acquired by an ultrasonic transducer were amplified and digitized by the same digitizer (100 MS/s). Figure 1 shows a schematic of the setup. At the time of the beginning of this study there was no reliable algorithm to quantify opto-acoustic signals in a way suitable for the analysis of single pulses. Automatic ceasings have been done with the optical feedback system only. The recorded data will be used to analyze the acoustic signals for a way of a single pulse analysis in simulations of the treatments.

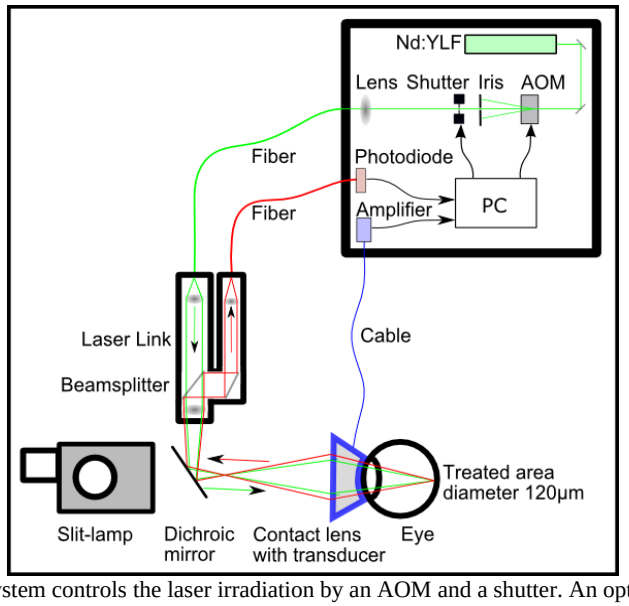

Figure 1 The Setup: A PC-system controls the laser irradiation by an AOM and a shutter. An optical system (laser link) projects a spot of desired size to the retina. Backscattered light is guided to a photodiode. Acoustic transients are acquired by an ultrasonic transducer. The optical and acoustical signals were analyzed for micro-bubble induced characteristics. The laser irradiation is ceased automatically upon bubble formation.

3. RESULTS

3.1 The Feedback Systems.

It has been achieved to create optical algorithm able to detect micro-bubbles by the analysis of one pulse in a burst of pulses. Figure 2 shows both quantified values (optical and acoustical) over the maximum radiant exposure of a pulse for one applied pulse train. It can be found that both values do correlate. A steep increase in found at an optical feedback value between 23 and 24. For the shown burst it refers to a maximum radiant exposure of around 280 mJ/cm². The differences in the trends vary. In the regime below threshold which is found in the optical feedback value 10 acoustic values show a small increase caused by ultrasonic signals of thermo elastic expansions and the optical signals vary randomly. In the regime above threshold the differences are characterized by a more random behavior of both methods.

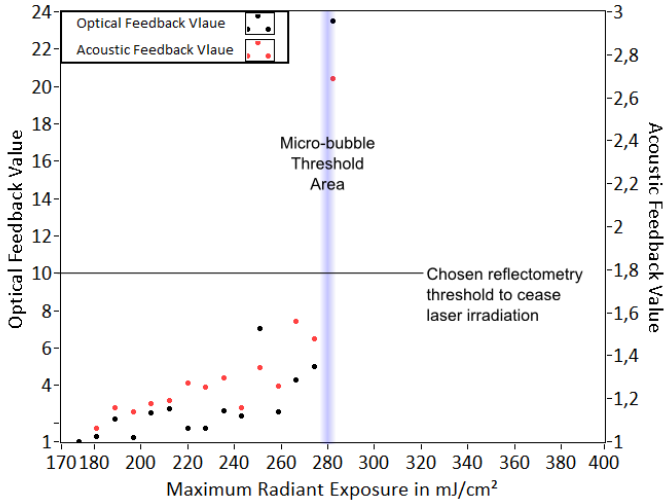

Figure 2 Quantification of micro-bubble induced characteristics of the optical and acoustical signals over the applied maximum radiant exposure.

Analyzing the automatic ceasings it can be seen that the required pulse energy to induce cell damage varies as predicted. The histogram (Figure 3) shows the number of all pulses in the maximum radiant exposure intervals where an automatic irradiation ceasing has been observed. A low threshold radiant exposure necessary to induce cell damage is characteristic for the rabbit fundus (Figure 3). The threshold pulse energy necessary for selective lesions varies locally (Figure 4).

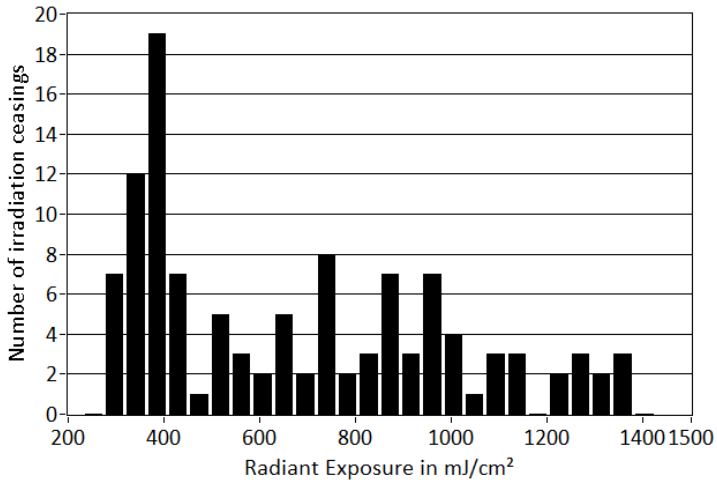

Figure 3 Histogram of ceasing maximum radiant exposures of various regions and eyes. All these radiant exposures have caused selective lesions at their specific region. The required radiant exposure at a specific region cannot be transferred to another region.

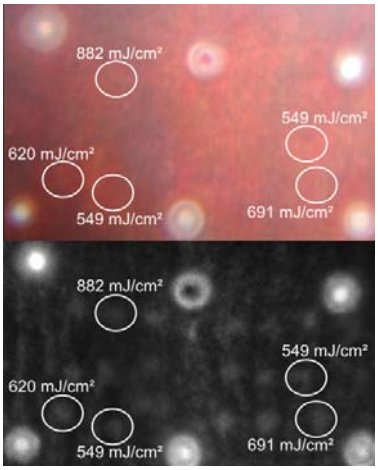

Figure 4 Variation of maximum radiant exposures; Top: Fundus image; Bottom FLA image

3.2 Selectivity, Healing and Safety

The first sign of selectivity is the invisibility in fundus images but visibility in fluorescence angiography (FLA) images. This is shown in Figure 5 and Figure 6. Figure 5 is a color photograph of the rabbit retina. It can be seen that the pigmentation varies from the upper part of the image (choroid is visible) to the center of the image (regions appearing black). The grid pattern of white spots on the retina is made by classical coagulations as marker spots. The grid is used for a better orientation during the treatment and evaluation. The SRT-Spots are placed inside the grid in a defined pattern. This makes it possible to find the areas in the FLA-images Figure 6). The fluorescent dye injected into the rabbits veins can pass through the RPE and Bruchs membrane initially. Thus the area appears as white spot in the FLA image (In the case of selective lesions the RPE cannot act as a blood-retina-barrier anymore). As in former studies the RPE is reconstructed one week after laser irradiation. In order to ensure that the selective lesions have not caused any damage to the neural retina OCT images have been performed after treatment. As can be seen in Figure 7 the classical photocoagulations at the marker sites have caused severe damage of the neural layers. The selective lesions have not caused any observable damage. A more detailed image of the treated areas has been obtained by H/E stained histology. The images Figure 8 and Figure 9 give an impression about the differences of classical photocoagulation and selective lesions. While the inner retinal layers have been destroyed by classical photocoagulation, the treated region at the SRT spots do not show any effect on the neural retina. The RPE itself is completely regenerated. In this case the RPE cells show some proliferations.

Figure 5 Color images of the rabbits fundus taken 1 hour after treatment show no visible lesions in the treated areas. Grid of white spots is made by classical coagulations for orientation during treatment.

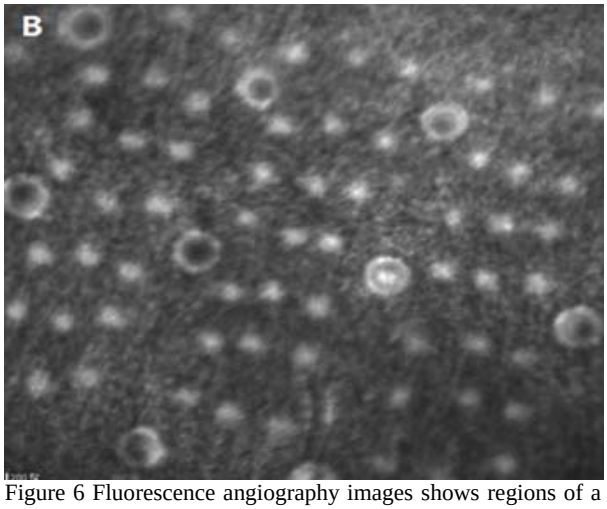

Figure 6 Fluorescence angiography images shows regions of a damaged blood-retina-barrier (RPE). Always 3x3 selective spots are included in a rectangle of marker lesions.

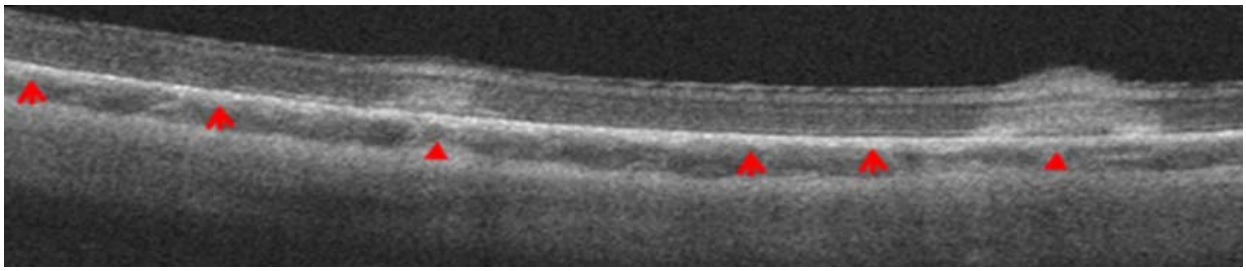

Figure 7 OCT images taken one hour after laser irradiation show an unaffected neural retina at the SRT spots (red arows) but severe damage at classical coagulation spots (red triangles).

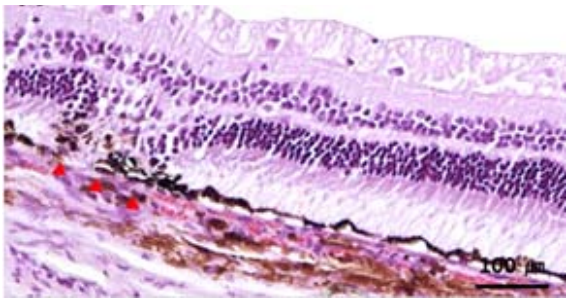

Figure 8 Histology image three weeks after treatment. Classical photocoagulation causes severe damage in the neural retina. Red triangles point to the location of the coagulation.

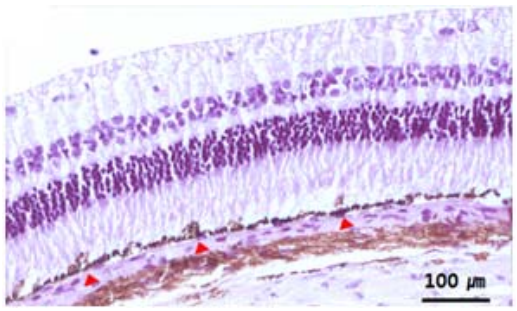

Figure 9 Histology image three weeks after treatment.
An intact neural retina and a fully recovered RPE can be found. In this case smaller focal proliferations of RPE cells can be found at the regions of the spots (red triangles).

4. DISCUSSION

The aim of this study was to evaluate the safety, selectivity and healing of the retinal lesions on rabbits by using an automatic irradiation control for SRT based on the evaluation of reflected light during irradiation. Automatic irradiation ceasings have been performed over a big range of maximum radiant exposures. The exposures leading to angiographic visibility and no ophthalmoscopic visibility (shown in the Histogram "Figure 10") have also been found above the statistical ED50 threshold for angiographic visibility which has been determined in former studies to 143 mJ/cm² with a 95 % confidence interval of 101 mJ/cm² to 170 mJ/cm². The radiant exposures of the confidence interval have been recalculated by the values given in [8]. The most likely reason for the difference of the statistical ED50 threshold to the thresholds found in this study is the technical difference of the setups and differences in the rabbits. In our study a lower repetition rate, a lower number of pulses per burst and an increasing energy within a burst have been applied. All these differences have the effect of an increasing threshold for RPE damage [2]. It shall be mentioned that not all bursts have been ceased immediately after the very first sign of bubble formation. Often a low number of pulses (2 to 3) have been applied with increasing energy after this threshold of bubble formation to get more distinct signals in the fluorescent angiography. Such delayed ceasings are safe as long as the radiant exposure stays inside the therapeutic window which is between the angiographic and ophthalmoscopic thresholds [9]. It is remarkable that selective lesions also occurred in regimes where ophthalmoscopic visibility is very likely. This is the regime above the ED50 threshold for ophthalmic visibility at around 478mJ/cm² [8]. Without a feedback system for monitoring or automatic irradiation control it would be very hard to work in this regime safely.

The selectivity and healing aspects in this study were very similar to findings in former studies. In the future it would be reasonable to test whether the observed focal proliferation of RPE cells may correlate with the strength \ size of the selective lesion or not and whether this can be controlled by the automatic irradiation control.

5. CONCLUSION

It has been shown that the developed optical feedback system detects micro-bubble induced characteristics in the backscattered light from the retinal pigment epithelium during selective retina therapy. The laser irradiation has been increased linearly until the quantified value reaches a predefined threshold value. The laser irradiation has been ceased as soon as the threshold was passed, no ophthalmoscopic visible lesions were produced. In conclusion the data look promising to realize a clinical SRT device based on this optical feedback technique. In a clinical device automatic SRT could shorten the treatment duration time.

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