Optoacoustic measurements during µs-irradiation of the retinal pigment epithelium

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ABSTRACT

The selective microphotocoagulation is a new technique to damage the retinal pigment epithelium (RPE), which is desired for treatment of several retinal diseases. By applying a train of µs laser pulses it is possible to selectively destroy these cells and simultaneously spare the adjoining photoreceptor and neural tissue. We applied µs laser pulses of a Nd:YLF laser (527nm), at a repetition rate of 500Hz to porcine RPE. The light is absorbed in the RPE and by thermoelastic expansion, an optoacoustic (OA) signal will be generated which could be measured by an ultrasonic transducer. With this setup, the baseline temperature increase at the RPE, during irradiation can be determined, since the optoacoustic pressure signal depends on the temperature of the irradiated RPE. We found a linear dependence of the OA amplitude to the RPE sample temperature. At higher irradiance we prooved the formation of microbubbles and bubble collapse in the RPE with OA techniques.

Keywords: optoacoustic, retinal pigment epithelium, selective photocoagulation, microbubbles, Grueneisen parameter, RPE

1. INTRODUCTION

The selective RPE cell treatment is a promising method in ophthalmology for a variety of diseases, which are associated with a dysfunction of the RPE cells[1]. In contrast to conventional methods for RPE cell destruction with cw (200ms) laser irradiation it allows to spare the photoreceptor tissue, thus maintaining full vision in the treated area. This allows the treatment in proximity of the fovea[1-2]. So far, 65 patients with different macular diseases have been treated with this new technique. The RPE cell destruction was proved by fluorescein angiography. Temperature calculations show[3], that during the laser pulse a high peak temperature will be generated at the absorbing melanosomes inside the RPE cells in the µs time scale (figure 1). Due to the thermal relaxation time of the melanosomes, heat could only penetrate over several µm. The high temperature at the melanosomes will damage the

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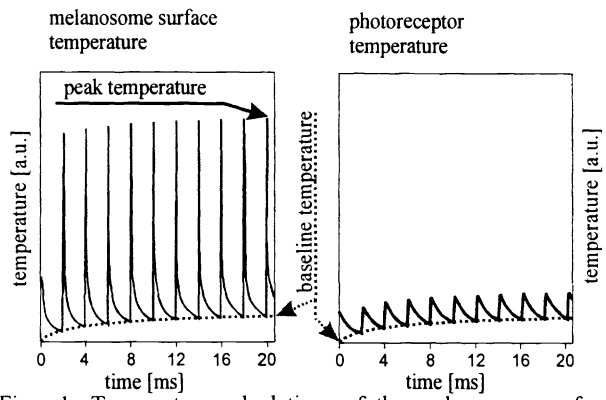

Figure1: Temperature calculations of the melanosome surface and the photoreceptor tissue during irradiation with repetitive µs laser pulses. During laser pulse high peak temperature will be generated at the mesanosome surface. At the photoreceptors, the temperature peaks are much lower due to the fact, that the laser pulses are shorter than the heat conduction time for the distance of 5µm. Induced by the train of laser pulses the baseline temperature increases in both cases.

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RPE cell. The temperature peaks at the adjacent photoreceptor are much lower. However, due to the high repetition rate, a baseline temperature is build up in the RPE. This effect is on the ms time scale, thus heat diffusion can reach other tissue layers like the photoreceptors as shown in figure 1. This makes the baseline temperature to an important factor, which interfere with the selective RPE cell destruction. Until now this baseline temperature increase was not measured although it is very important for the selectivity of the treatment.

The formation of microbubbles around the strong absorbing melanosomes inside the RPE has been reported for laser pulses shorter than 10^{-8} s in experimental[4] and theoretical studies[5-6]. In this case the RPE cell damage is most likely induced by mechanical effects such as cell membrane disruption[4] from the microbubbles or by laser induced stress waves[7]. For longer laser pulses (>10^{-4} s), the RPE cells are assumed to be thermally denaturated.

Objective of this study was to determine the baseline temperature increase and the formation of microbubbles in porcine RPE with optoacoustic techniques. These techniques have been used for temperature monitoring in canine liver[8] and for the proof of microbubbles around melanosomes[9].

2. MATERIAL AND METHODS

2.1 Setup

A frequency doubled, pulse stretched Nd:YLF laser[12] (527nm, 1.5µs pulse duration) was used as irradiation source. The fiber tip with top head beam profile was imaged with an ophthalmic laser slit lamp (Zeiss, 30 SL/L) to the sample which was fixed in a water filled cuvette (fig.1). Beam diameter at the sample was 156µm. The OA signals were received with an ultrasonic broadband transducer (Valpey-Fisher, VP-1093, 0-10 MHz) and recorded by a transient recorder (TEK/Sony, RTD710). The distance between sample and transducer was approximately 3 mm. The temperature inside the cuvette was measured near the sample by a thermocouple (Type J).

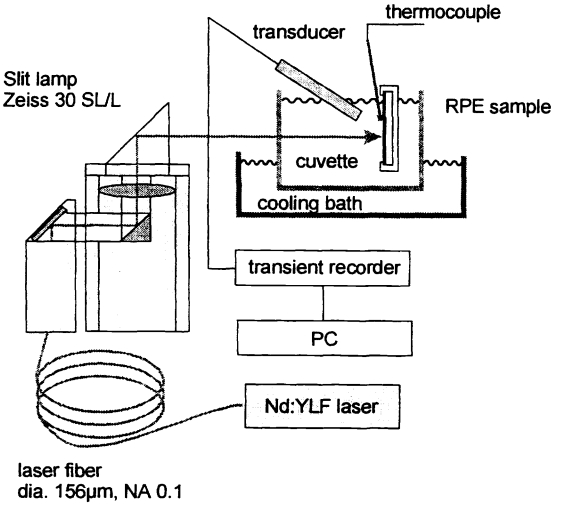

Figure 1: Setup for optoacoustic measurements during irradiation of porcine RPE with µs-laser pulses.

2.2 Optoacoustic techniques

Thermoelastic stress waves are based on thermal expansion of an energy absorbing medium. Under condition that the laser pulse duration is shorter than the thermal relaxation time of the target volume, the induced pressure P is given as[10]

$$P = \alpha \Gamma F_0 \quad (1)$$

with F_0 =radiant exposure, α =absorption coeff. , Γ = Grueneisen parameter of the absorbing medium. Γ can be expressed by the following material properties[10].

$$\Gamma = \frac{\beta}{\rho} C_v \kappa_\tau \quad (2)$$

with β =thermal expansion coeff., ρ = density, C_v = spec. heat capacity, κ_τ = isothermal compressibility. These parameters vary in dependence of temperature. For small temperature variations of several degrees Γ can be written as [11]

$$\Gamma(T) = \Gamma_0 + \Gamma_1 T \quad (3)$$

where T is the absolute medium temperature. In this case the pressure P goes linear with the temperature at constant radiant exposure.

$$P(T) = \alpha(\Gamma_0 + \Gamma_1 T) F_0 \quad (4)$$

There are some limitations to apply this to our experimental conditions. The laser pulse duration is in the order of the thermal relaxation time of the absorbing melanosome in the RPE cells. The assumption of an instantanious heating, which is needed for deriving equation *1* is not fulfilled. Also the acoustic relaxation time is much shorter than the laser pulse duration. During the laser pulse the pressure can propagate out of the heated volume and the resutling stress wave differs in amplitude and in shape. With our setup it is therefore only possible to measure the pressure signal in relative units. We have to add an experimental constant C_{exp} to equation *4* which include the transducer sensitivity, the distance between sample and transducer and the amplitude loss due to the unfulfilled acoustic confinement. Equation *4* could then expanded to

$$P_{exp}(T) = C_{exp}\alpha(\Gamma_0 + \Gamma_1 T)F_0 \quad (5)$$

At constant radiant exposure F_0, it can be reduced to two constants

$$P_{exp}(T) = A + BT \quad (6)$$

As we measure only the pressure in relative units it is not possible to calculate the absolute temperature form our OA data. However, it is possible to determine the baseline temperature increase based on the temperature dependence of the measured pressure signals.

From water it is known, that for temperatures exceeding 80°C the pressure increases less than linear with temperature, thus the Grueneisen parameter becomes a high order function of the temperature. High temperatures are also expected during irradiation of RPE. The absorbing melanosomes could heat up over 80°C at higher radiant exposures.

2.3 Sample preparation

The experiments were performed with enucleated porcine eyes. After equatorial dissection the vitreous gel, the neural, and the photoreceptor tissue were carefully removed. The sample with RPE as superficial layer was fixed in a holder system and covered with physiological saline solution.

2.4 Sample irradiation

For a calibration of the temperature dependence of the OA-signal on RPE, we measured the pressure signal for different temperatures of the PRE sample. The sample cuvette with warm physiological solution (45°C) was cooled down by the cooling bath. At constant radiant exposure of 50 mJ/cm², a repetition rate of 1 Hz, the optoacoustic pressure signal was recorded during cooling. A typical OA signal is shown in figure 3. The RPE sample temperature was measured with a thermocouple near the sample surface. As OA signal value we used the integral over the first positive bipolar wave to reduce noise from the pressure signal (fig. 3).

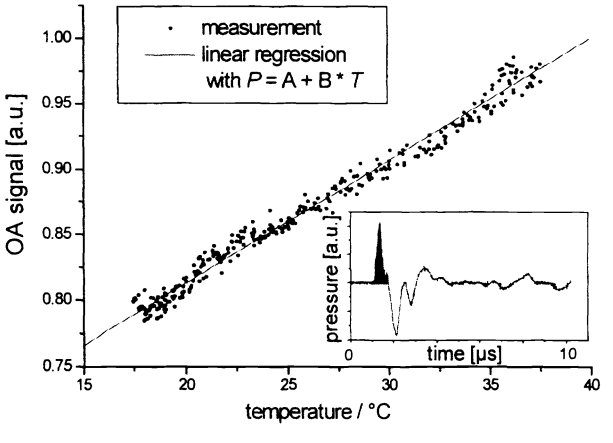

Figure 3: OA signal versus temperature. The pressure increases linear with temperature. Averaging over 17 samples with P=A+BT lead to values of A=-0.57(14) and B=0.0109(19). As OA signal value the integral over the positive bipolar wave was used to reduce noise from the optoacoustic signal.

The clinical used treatment parameter for selective photocoagulation are 100 laser pulses at repetition rate of 500 Hz. For this parameter it is important to know the increased baseline temperature induced by the train of laser pulses. During the first laser pulse, the temperature around the melanosome absorber increases. An optoacoustic signal will be generated. In the following time, the heat diffuses to the surrounding tissue, but a small temperature increase in the irradiated area remains, when the next laser pulse follows after 2 ms. This means an increased start temperature for this laser pulse, which leads to a higher optoacoustic signal at constant pulse energy, according to eq. *5*.

3. RESULTS AND DISCUSSION

3.1 Temperature dependence of the OA signal of porcine RPE

The OA signal emitted from irradiated RPE increases linear with the RPE temperature (fig. 3). Averaging over 17 samples leads to values of $A = -0.57 \pm 14$ and $B = 0.0109 \pm 19$ for the linear regression with $P = A + BT$. With this data as calibration values it is possible to calculate the temperature increase from the pressure increase during irradiation with a train of laser pulses.

As the OA signal is linear with temperature the heating of the melanosome at this radiant exposure is lower than 40 °C. A nonlinear dependence will be result if the melanosome temperature reach the nonlinear Grueneisen parameter region over 80°C.

3.2 Temperature measurements on RPE

At 500 Hz repetition rate, 1.5µs laser pulse duration and a radiant exposure of 160 mJ/cm² every single pressure signal was recorded. By analyze the OA signals, it is possible to calculate (eq. *6*) the temperature increase with the data from the calibration measurement.

As described above, the OA signal should increase with increasing pulse number due to the higher baseline temperature. This was also found experimentally (fig. 4). By analyzing every single OA signal from each laser pulse, it is possible to determine the temperature increase. The first pulse start at the sample temperature. With the known pressure-temperature dependence the following pressure amplitudes could directly converted to absolute temperatures.

The baseline temperature increased about 30 °C (fig. 5). At this radiant exposure the melanosome temperature is three times higher than in the pressure-temperature dependence experiment due to the threefold pulse energy. In this case a linear dependence of the Grueneisen parameter on the temperature could not securely be assumed. If the melanosome temperature is in the nonlinear region, the real baseline temperature increase is somewhat higher than calculated. In this case the results show a lower estimation of the real temperature.

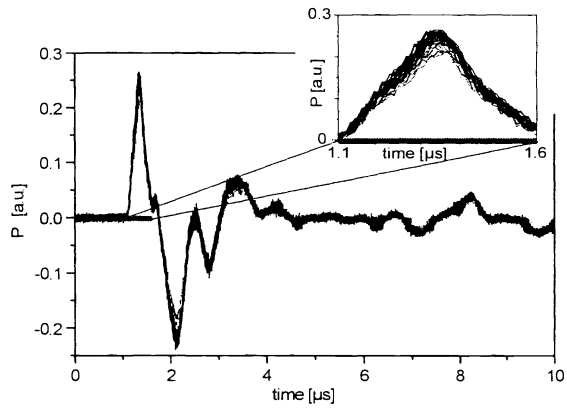

Figure 4: Superimposed optoacoustic signals during irradiation with a train of 100 laser pulses at rad. exp. of 160 mJ/cm² and 500 Hz repetition rate. The OA signal is a bipolar wave and very stable over the 100 pulses. Only the pressure maximum increases slightly, due to the baseline temperature increase of the RPE. In the magnification of the positive bipolar wave, the lower line is from the first laser pulse, and the topmost line from the last pulse.

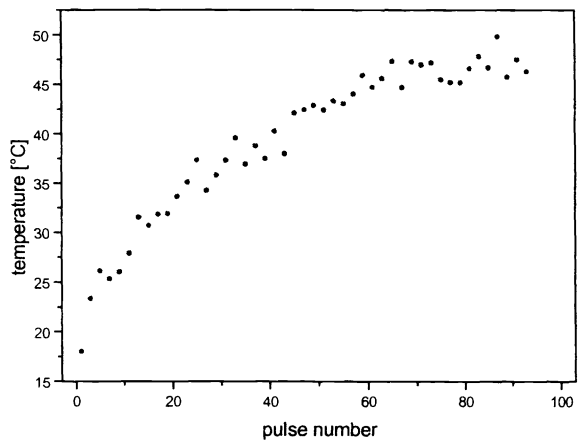

Figure 5: From the measured OA signal calculated baseline temperature increase of irradiated RPE. (100 laser pulses, 500Hz repetition rate, 160 mJ/cm², 156 µm spot size). The final temperature increase is approximately 30 °C.

3.3 Formation of microbubbles in RPE cells

Increasing the radiant exposure to 300mJ/cm² a disturbance at the end of the bipolar optoacoustic wave appears (fig. 6). This can be interpreted as the formation of microbubbles around the absorbing melanosome. Slightly above the threshold radiant exposure, the microbubble formation could not be determined from a single OA signal. The disturbance of the thermoelastic bipolar signal is very weak. Only by superimposing several OA signals the formation could be visualized. This is comprehensible, as we irradiate simulaneously 500 RPE cells with about 200 melanosomes inside each cell. At the microbubble threshold we have a large pure thermoelastic bipolar signal from the main part of the 100.000 melanosomes and the bubble formation signal of probably several microbubbles. Therefore also the disturbance did not start very significant as known from other optoacoustic bubble collapse experiments[13].

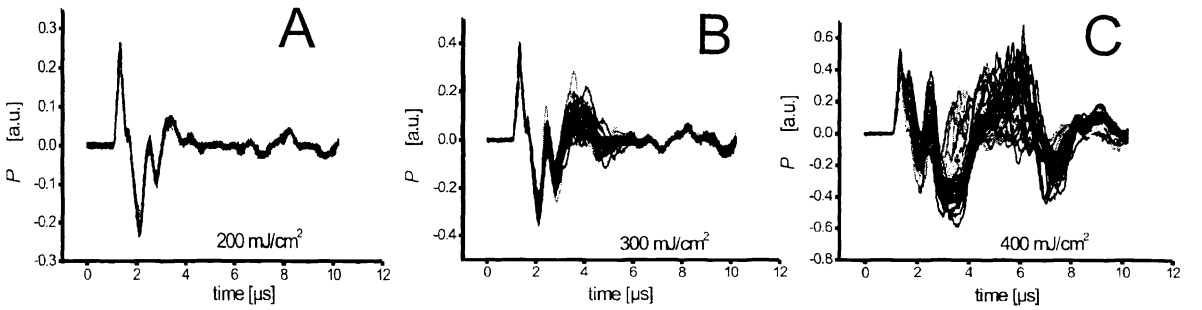

Figure 6: Change of the OA signal with increasing radiant exposures. The 100 optoacoustic signals from the laser pulse train were superimposed.
A: Radiant exposure 200mJ/cm². The signal is pure thermoelastic.
B: Radiant exposure 300mJ/cm². At the end of the thermoelastic bipolar wave signal fluctuations appear, which are explained by the formation of microbubbles.
C: Radiant exposure 400mJ/cm². The signal deviations from the bubbles are larger than the thermoelastic signal.

At higher radiant exposure of 400 mJ/cm² the disturbance is higher than the thermoelastic bipolar wave. At 450 mJ/cm² a second peak is observed, which can be interpreted as a bubble collapse (fig. 7). With increasing pulse number the delay time between the first pressure peak and the collapse signal increases. An increasing bubble size due to the higher baseline temperature at higher pulsenumber can explain this observation. We measured delay times up to 35µs at even higher irradiations.

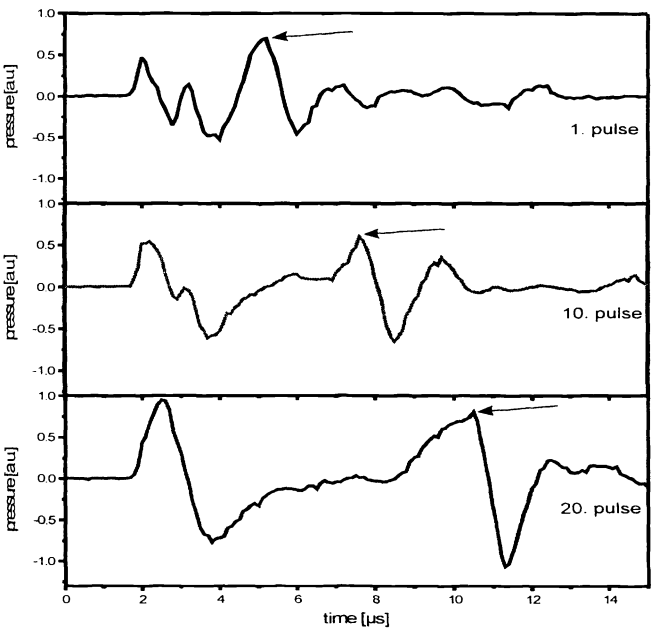

Figure 7: Optoacoustic signals at radiant exposure of 450 mJ/cm². After the bubble formation signal (first positive peak) a secound pressure peak (arrow) appears which could be assumed as bubble collapse. The delay time between bubble formation signal and collapse signal increase over the number of pulses.

3.4 Optoacoustic measurements during treatment

During clinical treatment a contact glass is placed on the patient's cornea to couple the laser radiation into the eye. We modified a standard contact glass with a piezzo electric transducer. With this optoacoustic contact glass we were able to measure the OA signals during treatment (fig. 8). The represented signals were measured at a radiant exposure of 600 mJ/cm² with a retinal spot diameter of 200 µm. In this first experiments, a pressure peak increase due to a baseline temperature increase was also found.

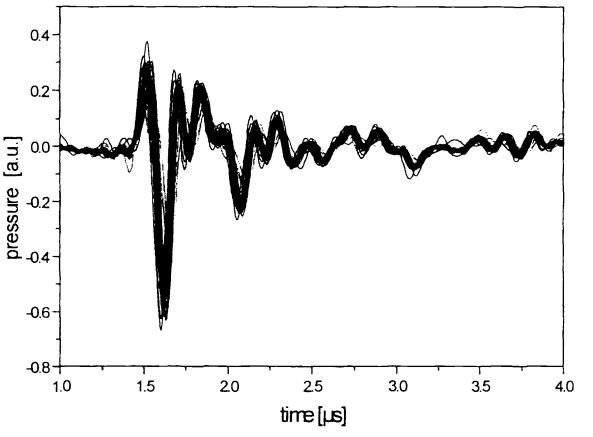

Figure 8: Measured OA signals during patient treatment. The pressure peak increases with pulse number, compareable to the in-vitro measurement.

4. CONCLUSIONS

With optoacoustic techniques it is possible to determine the baseline temperature increase in the RPE during irradiation with a train of laser pulses. The formation of microbubbles and the bubble collapse in porcine RPE was also determined optoacoustically. Using an optoacoustic contact glass, measurements can also be performed in vivo during treatment. This will lead to a better understanding of the effects involved in the selective photocoagulation of the RPE.

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