Non-invasive Monitoring of the Thermal Stress in RPE Using Light Scattering Spectroscopy


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ABSTRACT

Introduction: Light Scattering Spectroscopy has been a recently developed as a non-invasive technique capable of sizing the cellular organelles. With this technique, we monitor the heat-induced sub-cellular structural transformations in a human RPE cell culture.

Material and Methods: A single layer of human RPE cells (ATCC) was grown on a glass slide. Cells are illuminated with light from a fiber-coupled broadband tungsten lamp. The backscattered (180 degree) light spectra are measured with an optical multichannel analyzer (OMA). Spectra are measured during heating of the sample.

Results: We reconstructed the size distribution of sub-micron organelles in the RPE cells and observed temperature-related changes in the scattering density of the organelles in the 200-300nm range (which might be peroxisomes, microsomes or lysosomes). The sizes of the organelles did not vary with temperature, so the change in scattering is most probably due to the change in the refractive indexes. As opposed to strong spectral variation with temperature, the total intensity of the backscattered light did not significantly change in the temperature range of 32-49 °C.

Conclusion: We demonstrate that Light Scattering Spectroscopy is a powerful tool for monitoring the temperature-induced sub-cellular transformations. This technique providing an insight into the temperature-induced cellular processes and can play an important role in quantitative assessment of the laser-induced thermal effects during retinal laser treatments, such as Transpupillary Thermal Therapy (TTT), photocoagulation, and Photodynamic Therapy (PDT).

Keywords: Light Scattering Spectroscopy, LSS, retinal pigment epithelium, RPE, Transpupillary Thermotherapy, TTT, cell sizing, optical diagnostics, on-line, dosimetry, thermal stress

1. INTRODUCTION

Many recent developments in the treatment of retinal diseases are focused on targeting of the diseased fundus structures. For a large patient group with age related macular degeneration (AMD), the choroidal neovascularizations are the target structures in such procedures as photo dynamic therapy (PDT) and transpupillary thermo therapy (TTT). Especially for TTT, a purely thermal treatment modality, the inter- and intra-individual variability of the ocular transmission, retinal absorption and choroidal blood perfusion makes a treatment outcome nearly unpredictable. A real time dosimetry system is required for TTT in order to monitor the laser-induced retinal heating at the levels below the irreversible cellular damage.

Two different approaches to monitoring of the laser-induced retinal temperature increase are currently under development. One of them is based on intravascular injection of the thermosensitive liposome which can release its fluorescent content in retina when heated above certain threshold temperature. The disadvantages of this method are in its invasive character of the systemic injection of large amounts of dye and in the limited information about the
maximal temperature in RPE. In the second approach, the temperature dependence of the thermal expansion coefficient of the retinal pigment epithelium (RPE) is used to probe the temperature using an optoacoustic approach. In this technique a short-pulsed laser has to be applied to the fundus in addition to the treatment laser. Increase of the light scattering from the retina during coagulation has been used for monitoring and dosimetry of the laser photocoagulation. In this technique, the change of the light scattering was due to the thermal denaturation of the neurosensory retina. During TTT the neurosensory retina should not be adversely affected and certainly not denatured. The therapeutic strategy of the TTT is selective destruction of the choroidal neovascularization achieved by localized heating of RPE. Sub-cellular transformations in the thermally stressed cells involve the expression of the heat shock proteins, in particular in the RPE cells.

Light scattering spectroscopy (LSS) is a novel technique which allows for non-invasive measurement of optical properties and dimensions of the sub-cellular structures. This technique has been used successfully for the optical detection of the dysplastic changes in epithelial tissues.

In this study we apply LSS for monitoring the temperature-induced sub-cellular transformations in the RPE cells.

2. MATERIAL AND METHODS

2.1. LSS setup

All LSS spectra were collected using the experimental setup shown in Fig. 1. The light of a 100-W halogen lamp was coupled to a 3mm fiber bundle. The fiber tip was imaged through a 50% beamsplitter onto the sample chamber. The direct back-scattered light (180 ± 5 deg.) from the sample passing through the 50% beamsplitter was coupled to an optical multichannel analyzer (OMA) via a microscope. The sample chamber was placed on a mount with a temperature gradient. By moving the mount with the sample chamber using a translation stage, different temperature courses have been measured within one sample. The data acquisition process and the sample movement were controlled by a PC.

2.2. RPE samples and experiments

Unpigmented human RPE cells (ATTC) were grown as a monolayer on a glass slide. For the experiments, the samples were placed on the preheated mount. During heating the scattering spectra at five different locations on the sample were probed. For calibration purposes the temperature-time courses for these five locations were first measured with a micro thermocouple (Fig. 2). The maximum temperatures at these locations range from 39 °C to 47°C. After the spectral measurement with heated cells, the life/dead fluorescent assay (CalzeinAM & Iodithium Bromide, Molecular Probes) has been applied to assess the cell survival.
2.3. Reconstruction of the scatterers size distribution from the measured scattering spectra

Mie-scattering calculation in the geometry of the optical setup was used to calculate the scattering spectra for spheres with diameters ranging from about 100 nm to several micrometers. Weight coefficients for each scatterer size were then determined by the best fit to the experimental spectra. With this method, the scatterer size distribution of the sample can be extracted from the measured light scattering spectra.

3. RESULTS AND DISCUSSION

3.1. Calibration with polystyrene bead mixtures

To prove that the sizes of several sub-cellular scatterers can be extracted from the scattering spectrum, experiments with mixtures of spherical polystyrene (PS) beads were performed. A mixture of 5 different PS bead sizes (291nm, 585nm, 737nm, 1053nm, 2105nm) was diluted in water at concentrations ratio of 5 : 5 : 1 : 1 : 1. A scattering spectrum of this mixture is shown in Figure 3.

The scatterer size distribution of the PS bead mixture (Figure 4) was reconstructed by analyzing the scattering spectrum (fig. 3) with the method described above (section 2.3). As one can see in Figure 4, the bead sizes and their relative concentrations can be accurately extracted from the scattering spectrum. One false peak appears close to the biggest bead size (2105nm). It is remarkable that small structures are clearly resolvable far below the optical resolution of a conventional light microscope.

3.2. RPE light scattering spectra

Two typical RPE scattering spectra at different temperatures are shown in Figure 5. The spectral shape seems to be dominated by the Rayleigh scattering with the wavelength dependence of $1/\lambda^4$. Depending on the temperature-time course applied to the cells the light scattering spectra where found to change quite significantly, as shown in Figure 5. The light scattering increases towards the long wavelength region. Life/dead staining indicated no cellular death at these temperatures.
3.3. Scatterers size distributions in RPE cells

Distributions of the scatterers sizes in the range of 100-1600nm (Figure 6) have been extracted from the RPE scattering spectra measured at different temperatures (shown in Figure 5). From these spectra, four very distinct scatterer sizes have been obtained. The sub-micron scatterers can be associated with the cellular organelles such as microsomes, mitochondria or protein vacuoles. A more detailed TEM study is under way to measure the sizes of the organelles in the RPE cells.

It appears from Figure 6 that not the sizes of the scatterers change with temperature, but rather their scattering density, which indicate changes in the refractive index of the organelles under thermal stress.

3.4. Monitoring the temperature induced sub-cellular transformations in RPE

One can monitor the cellular stress, for example, by following the integral value of the smallest scatterer size (150-300 nm) during the heating process, as shown in Figure 7. All the cells were alive within the temperature range of 39°C to 47°C. The scatterer density significantly increases at temperatures exceeding 43 °C. This result is in good agreement with the literature value for the expression of heat shock proteins11. The scatterer density is proportional to the scatterer concentration and to their relative refractive index. Since the organelles can’t multiply, the rise in scattering is most probably due to the increase of their refractive index. At 47°C the density increases very significantly - by 60% of its original value.

![Figure 6: Scatterer size distributions of heated and non-heated RPE cells. The scatterer density (i.e. refractive index) but not the sizes of the organelles seem to change under thermal stress.](image)

4. CONCLUSION

We have demonstrated that the Light Scattering Spectroscopy can be successfully used for monitoring the temperature-induced sub-cellular transformations. Based on an inverse light scattering analysis method the scatterer sizes and densities can be extracted from the measured light scattering spectra. The technique has been successfully tested on a mixture of polystyrene beads. Applying this technique to the scattering spectra acquired from the human RPE cell cultures during heating, we were able to monitor the early phases of the temperature-induced transformations. This technique, providing an insight into the cellular metabolic processes induced by the thermal stress, can play an important role in quantitative assessment of the laser-induced thermal effects during retinal treatments, such as Transpupillary Thermal Therapy (TTT), cw photocoagulation. This technique might also allow for monitoring of the PDT treatment process, as significant metabolic effects should occur during the procedure.

REFERENCES


