Kapitel 6

Summary

Photodynamic therapy (PDT) is a treatment for superficial tumorous and dysplastic tissue in which light is used to excite photosensitizers within tissue to transform molecular oxygen, via energy transfer processes, into oxygen radicals. As the accumulation of the sensitizers takes place in the tissue selectively, the cytotoxic reaction of the radicals which destroy the surrounding tissue only occurs locally. The effect of PDT is based on the transformation of molecular oxygen into radicals, so that exact knowledge regarding the oxygen concentration in tissue is crucial to the success of the therapy. An oxygen monitoring system is therefore required.

The molecular oxygen concentration can be calculated by measuring the luminescence lifetime of dyes by making use of selective oxygen-sensitive luminescence dyes used in optical molecular imaging to diagnose diseases and processes. The luminescence lifetime of theses dyes is shortened by energy transfer processes dependent on the concentration of quencher molecules. This means that knowledge of the lifetime characteristic can be used to evaluate the quencher concentration. The process is called dynamic quenching and is described by the Stern-Volmer-equation. The correlation between lifetime and quencher concentration is quantified by the Stern-Volmer-constant which is dependent on the diffusion coefficients of molecule and quencher, and therefore, dependent on the temperature and solvent. Molecular oxygen is an example of a quencher for luminescent ruthenium-complexes. The luminescence lifetime can be calculated pixel-wise with the Rapid-Lifetime-Determination-method (RLD) and a CCD-camera, making it possible to calculate an oxygen concentration image.

Within the framework of this work a complex, but nevertheless modular, luminescence lifetime imaging system was constructed with which it is possible to calculate and display the lifetime and the oxygen concentration, both one- and two-dimensionally, using a CCD-camera and the RLD-method. This made monitoring of the oxygen concentration possible whereby up to 6 $images/min$ could be displayed and the colored labeling of the relevant oxygen concentration followed automatically. Two dyes, Ruthenium-tris-bipyridyl ($Ru(bpy)_3^{2+}$)

dissolved in aqueous media and the commercially available oxygen-permeable Sol-Gel-layer FOXY-SGS-M with an embedded ruthenium-complex were used to test the system. Time-consuming temperature- and oxygen concentration measurements were carried out using two different cuvettes: a flow-through cell and a calibration cell, both specially constructed for this work. The functionality of the system was proved by comparing the measured Stern-Volmer-constant of $Ru(bpy)_3^{2+}$-solutions with values from the literature, which showed very good agreement.

Dependence of the lifetime of the $Ru(bpy)_3^{2+}$-solution and the FOXY-layer on pH and temperature in the two cells was evaluated. The results showed that there was no dependence on pH, but that there was a temperature dependence which necessitated in situ measurement of temperature or the temperature stability of $\pm 1\,^{\circ}C$ for the $Ru(bpy)_3^{2+}$-solutions. The temperature dependence of the FOXY-layer is smaller, requiring only a temperature stability of $\pm 3\,^{\circ}C$. The sensitivity of the dyes was at its greatest in the range of low oxygen concentrations i.e. relevant for PDT oxygen whereby that of the FOXY-layer is smaller than $Ru(bpy)_3^{2+}$ despite the longer lifetime. The *in vitro* measurements using pig skin samples and the dye $Ru(bpy)_3^{2+}$ showed that not only the dye lifetime is changed after contact with the skin but also the dynamics of the lifetime change are decreased in the relevant concentration range. Therefore one can assume that the dye is unsuitable for *in vivo* measurements. However further experiments must be carried out in order to confirm this behavior *in vivo*. The lifetime and dynamics of the FOXY-sensor showed no change due to the contact with the pig skin. The response times of both dyes were comparable to established oxygen sensors making them suitable for oxygen concentrations measurements. The FOXY-layer appears to be ideal for minimal invasive measurement of tissue because it has a short response time, is independent of pH, and has a low temperature dependence. Furthermore due to only superficial contact, it is presents no danger to humans and does not adversely affect PDT.

The realized luminescence lifetime measuring system is highly suitable for two-dimensional oxygen concentration analysis of probes and tissue and presents a key step forward to a monitoring technique for PDT. Determination of the oxygen concentration in tissue and whether the progress of therapy can be evaluated by the superficial measurement will be the aim of further research projects. The examination of different markers is possible because the system is built up of modules. Other application areas could therefore be molecular-medicinal problems such as testing target specificity of newly developed anchor molecules for contrast or therapeutic agents