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The Effect of Venous and Arterial Occlusion of the Arm on Changes in Tissue Hemodynamics, Oxygenation, and Ultra-Weak Photon Emission

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Abstract: Ultra-weak photon emission (UPE) is a general feature of living biological systems. To gain further insights into the origin of UPE and its physiological significance, the aim of the present study was to investigate the connection between hemodynamics (HD), oxygenation (OX), and UPE. Therefore, during venous and arterial occlusion (VO, AO), changes of UPE and surrogates of HD as well as OX were measured simultaneously using two photomultipliers and near-infrared spectroscopy, respectively. We showed that (1) changes in UPE correlate significantly nonlinearly with changes in oxyhemoglobin ($\Delta[O_2Hb]$), deoxyhemoglobin ($\Delta[HHb]$), and hemoglobin difference ($\Delta[HbD] = \Delta[O_2Hb] - \Delta[HHb]$), indicating a complex association between UPE and tissue HD/OX; (2) UPE decreases significantly during AO but not during VO; (3) UPE increases significantly after AO; and (4) the view that ROS are the source of UPE is generally supported by the present study, although some findings remain unexplained in the context of the theory of ROS-mediated UPE generation. In conclusion, the present study revealed new insights into the interplay between HD, OX, and UPE and opens up new questions that have to be addressed by future studies.

Keywords: Blood flow · Blood volume · Oxygenation · Oxidative stress · Ultra-weak photon emission · Reactive oxygen species

1. Introduction

Living biological systems spontaneously emit ultra-weak light (~10^2 photons/s cm^2 [1]) in the wavelength range of at least 200–800 nm from their surface. Previous experiments have shown that this ultra-weak photon emission (UPE) is associated with the relaxation of electronically excited states of molecules, e.g., lipids [2] and proteins [3]. The excited states are mainly consequences of oxidation reactions caused by reactive oxygen species (ROS). Reactions involving singlet molecular oxygen ($^1O_2$) [4] and triplet states of carbonyl [3] have been identified as causing UPE in particular. Early studies have demonstrated that UPE of isolated organs is highly oxygen dependent [5]. The decrease of UPE after application of antioxidative enzymes [6] supports the findings of ROS-based UPE generation.

Up to now, only a few studies [7–11] have investigated the effect of a temporally local shortage in blood supply (ischemia) and blood oxygenation (hypoxia) on UPE of intact organisms, some of these focusing on humans. To gain further insights into the interplay between UPE dynamics, changes in hemodynamics (HD) (i.e., blood flow/volume) and oxygenation (OX), the aim of the present study was to measure UPE, surrogates of HD and OX simultaneously for the first time on a human using two photomultipliers and near-infrared spectroscopy (NIRS).

2. Material and Methods

2.1. NIRS and UPE Measurement

Surrogates of HD and OX were measured using a wireless NIRS device [12]. The NIRS device uses LEDs with two different wavelengths (760 and 870 nm) to deter-mine absorption changes with a sampling frequency of 100 Hz. For the current study we selected four light paths (source-detector separation: 25 mm) from the device. The area of the probed tissue was 25 × 37.5 mm in total.

The UPE measurement was performed using a specially designed dark chamber with two compartments for recording on both human hands. On top of each chamber a single-photon counting photomultiplier tube (PMT) (Electron Tube 9235B, Electron Tubes Ltd., Ruislip, UK) was placed. The spectral sensitivity of the PMT was 200–650 nm. Subjects were dark-adapted and then inserted their left and right hand into the compartments of the dark chamber. The measurement was controlled using Labview (National Instruments, Austin, USA).

2.2. Measurement Procedure

Two healthy male adults participated in the study (ages: 28 and 44 year). Prior to the measurement, the hands of the subjects were covered for 45 min with black gloves to reduce delayed luminescence [13]. The subjects then positioned their hands in the dark chamber. UPE of the palm side was measured. This side was selected for measurement because commonly it has stronger emission than the dorsal side of the hand [14, 15]. The NIRS optode was placed on the left forearm, in parallel to the *flexor digitorum profundus* muscle, ~7 cm below the crook of the arm. A blood pressure cuff was placed around the left upper arm. The experimental setup is shown in Fig.1. The measurement protocol was as follows: (1) 0–6 min: baseline recording; (2) 6–11 min: venous cuff occlusion (VO) (at 40 mmHg); (3) 11–21 min: reperfusion; (4) 21–31 min: arterial occlusion (AO) (at 200 mmHg); (5) 31–40 min: reperfusion.

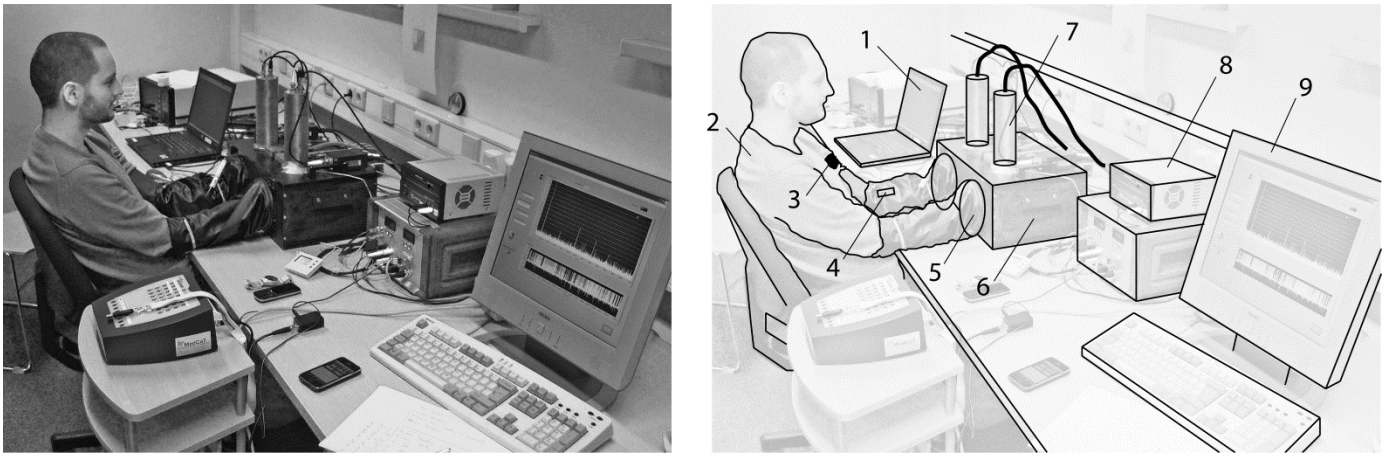

Fig. 1 Measurement setup. 1: near-infrared spectroscopy (NIRS) data recording, 2: subject, 3: occlusion cuff, 4: attached NIRS device, 5: light shielded opening of the dark chamber to insert the hand, 6: dark chamber, 7: PMT, 8: power supply and PMT controlling electronics, 9: PMT controlling and measurement.

Three measurements were made in total (i.e., one subject was measured twice).

2.3. Signal Processing and Data Analysis

All calculations were performed using Matlab (Mathworks, Natick, Massachusetts, USA). The UPE signal ($I_L(t)$) from the left hand was corrected by subtracting the dark count of the PMT ($I_D(t) = 6$ counts/s (cps)): $I_L'(t) = I_L(t) - I_D(t)$. Afterwards, the sum of all values of $I_L'(t)$ for every minute of recording time was calculated and all obtained values were divided by 60, leading to the final signal $I_L''(t) = \{I_L'(t_i)\} \mid i = 1, 2, \ldots, 40\}$ which contained the corrected UPE signal of the left hand, expressed in "cps."

From the absorption changes measured using NIRS, concentration changes in μM (relative to the first value obtained by the measurement) of oxyhemoglobin ($\Delta[O_2Hb]$) and deoxyhemoglobin (Δ [HHb]) were calculated for every light path using the modified Lambert-Beer law [16]; the differential pathlength factor (DPF) was 4.48 (for 760 nm) and 3.81 (for 870 nm) [17]. Additionally, the following signals were calculated: total hemoglobin concentration ($\Delta[tHb] = \Delta[O_2Hb] + \Delta[HHb]$) and hemoglobin difference ($\Delta[HbD] = \Delta[O_2Hb] - \Delta[HHb]$). $\Delta[O_2Hb]$ and $\Delta[HHb]$ are associated with HD and OX, $\Delta[tHb]$ primarily with HD (especially blood volume changes [18]), and $\Delta[HbD]$ with OX changes [19].

All NIRS signals were then downsampled to a sampling frequency of 1 Hz and smoothed using robust local scatterplot smoothing (LOESS) with a window size of 30 s to get rid of physiological noise (such as heart pulsation and Mayer waves). Finally, for all the four signals ($\Delta[O_2Hb]$, $\Delta[HHb]$, $\Delta[tHb]$, and $\Delta[HbD]$), the mean signal was calculated by averaging the signals from all four light paths having the same source-detector separation of 25 mm.

For further analysis, the NIRS signals and the UPE signal (of the left hand) from all three experiments were averaged leading to one signal each. Additionally, the mean values for 60-s long segments of the signals were calculated. To reduce the variance in the averaged UPE signal, the UPE signals were normalized so that that the average of each signal was equal to the average of all signals. The UPE signal of the right hand was not used for further analysis since it was recorded only as a control signal.

Two types of analysis were performed: (1) significance of NIRS and UPE changes depending on the 5 phases of the experiment, and (2) correlation between NIRS and UPE signals and their significance thereof. For the correlation analysis we empirically determined the mathematical function with the best fitting and we calculated the Pearson correlation between the fitting function and the NIRS and UPE signals. Unconstrained nonlinear minimization of the sum of squared residuals was used for the fitting. For calculating the p-values of the changes a Wilcoxon rank sum test was used. The p-values for the regression coefficients were computed using a Student's t distribution for a transformation of the correlation.

3. Results

Figure 2a shows the time courses of the UPE, $\Delta[O_2Hb]$, $\Delta[HHb]$, $\Delta[tHb]$, and $\Delta[HbD]$ signals obtained. It is evident that NIRS signals between experiments differ only slightly and are consistent between experiments. In contrast, for UPE it is evident that relatively larger and more complex fluctuations occur which may obscure small VO and/or AO related changes. The following significant ($p < 0.05$) changes were observed: During the VO, $\Delta[HHb]$and $\Delta[tHb]$ increase, $\Delta[HbD]$ decreases. After the VO, i.e., the first reperfusion phase, $\Delta[HHb]$, $\Delta[tHb]$, and $\Delta[HbD]$ return to baseline values. During the AO, $\Delta[O_2Hb]$ and $\Delta[HbD]$ decrease, $\Delta[HHb]$ and $\Delta[tHb]$ increase, UPE decreases above the baseline level. After the AO, i.e., the second reperfusion phase, $\Delta[O_2Hb]$, $\Delta[tHb]$, and $\Delta[HbD]$ show a transient increase, and $\Delta[HHb]$ a transient decrease. At the same time, UPE increases slowly.

Figure 2b shows the averaged signals that were used for the correlation analysis. It was found that the UPE and NIRS signals showed a correlation when an exponential function of the form $y = \alpha[\beta \exp(\gamma x)]^{-1}$ with three free parameters (α, β, γ) was used for correlation analysis. UPE correlates significantly ($p < 0.001$) with $\Delta[O_2Hb]$, $\Delta[HHb]$, and $\Delta[HbD]$: $r(\text{UPE}/\ \Delta[O_2Hb]) = 0.7182$ ($p = 1.8146 \cdot 10^{-7}$), $r(\text{UPE}/\Delta[HHb]) = -0.7431$ ($p = 3.989 \cdot 10^{-8}$), and $r(\text{UPE}/\ \Delta[HbD]) = 0.7425$, ($p = 4.1513 \cdot 10^{-8}$). The correlation between UPE and $\Delta[tHb]$ was not significant $r(\text{UPE}/\Delta[tHb]) = -0.2229$ ($p = 0.4668$).

4. Discussion, Conclusion, and Outlook

The changes observed in $\Delta[O_2Hb]$, $\Delta[HHb]$, $\Delta[tHb]$ and $\Delta[HbD]$ during and after the occlusions are in agreement with previous studies (e.g., [19]).

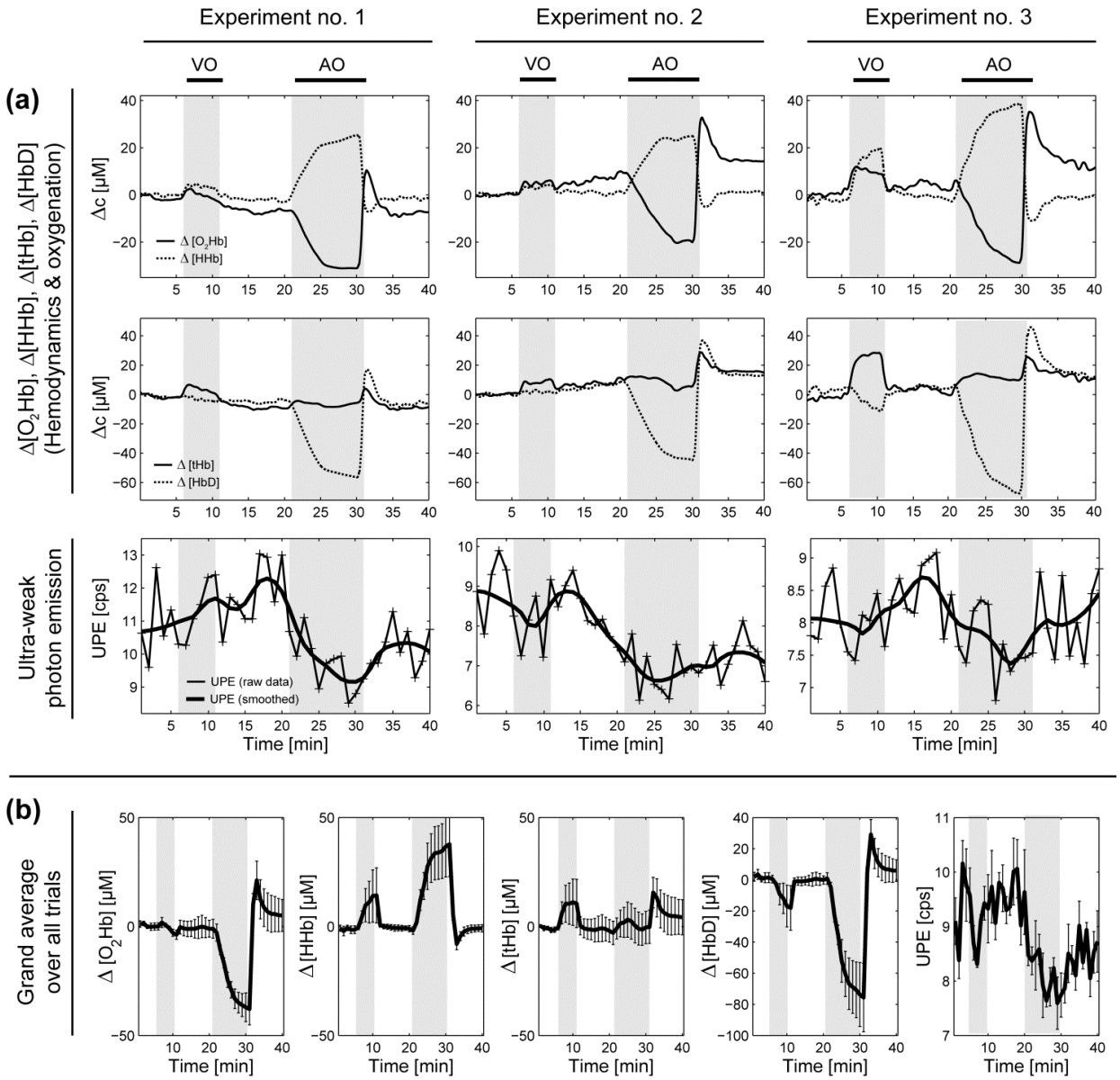

Fig. 2 Overview of the measured Δ[O_2Hb], Δ[HHb], Δ[tHb], Δ[HbD], and UPE signals before, during, and after VO and AO on the forearm. (a) Shows the signal from each experimental trial, (b) depicts the averaged signals and standard errors from all three measurements.

The UPE changes agree, to some extent, with findings from other studies: (1) A decrease in UPE during AO was reported by [7, 10, 11], in agreement with our results. It is also in agreement with findings of [8] who found a decrease of UPE in brain tissue after cardiac arrest of a rat, indicating that hypoxia is associated with a UPE decrease. (2) A smaller decrease in UPE during VO (compared to AO) on human subjects was reported by [10, 11]; the decrease in UPE measured in our study was not significant. (3) A return of UPE to baseline level in the reperfusion phase was observed by [10, 11], partially in agreement with our results since UPE increased in our study after the AO in the second reperfusion phase but not reached the baseline level. Our results also agree with findings that during hyperoxia (by changing the oxygen of the inhaled air), brain tissue of the rat showed an UPE increase above baseline level [9]. A peak in lucigenin-enhanced chemiluminescence (corresponding to an increased $^1O_2$ production) in the reperfusion phase after hypoxia in rat brain tissue was found in another study [20].

The finding that UPE correlates significantly with Δ[O_2Hb], Δ[HHb], and Δ[HbD] in a nonlinear manner indicates a complex relationship between UPE and HD/OX. Regarding the molecular processes underlying the correlation, changes in ROS concentration could be a factor linking the two. However, there are some discrepancies: (1) previous studies showed an increase in ROS during *hypoxia* (occlusion) [21, 22]; consequently, hypoxia should increase UPE which contradicts the results of our study and observations [7, 8, 10, 11]. Though, on the one hand, ROS formation depends on oxygen while, on the other, ROS are generally formed under various stress conditions. In the situation examined here, hypoxia may initially lead to limited oxygen supply and decrease in ROS formation, while an extended period of hypoxia may result in stress which then results in enhanced ROS formation.

During *hyperoxia* (reperfusion), a strong increase in ROS occurs: the enzyme xanthine oxidase reacts with O_2 leading to the formation of ROS (especially superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$)) [23]. Consequently, UPE is expected to strongly increase during reperfusion. Indeed, we found an increase (albeit a relatively small one) while other studies [10, 11] did not.

The exact origin of UPE measured from the hand is unknown. It has been estimated that about 60% of the UPE is based on internal sources. The rest may be attributable to oxidative processes on the skin surface [24].

Since the present work was only a pilot trial, there were certain limitations that need to be addressed in further

studies: (1) small sample size and number of trials, (2) relatively short rest periods between the occlusions, (3) no direct measurement of OX (the usage of a multidistance NIRS algorithm is suggested), and (4) measurement of UPE and HD/OX at different muscle compartments of the arm. Further studies should extend the analysis of the relationship between UPE and HD/OX – especially since UPE could deliver some new information about hemodynamic and metabolic changes in tissue and the primary source of UPE.

In conclusion, we showed that (1) changes in UPE correlate significantly nonlinearly with $\Delta[O_2Hb]$, $\Delta[HHb]$, and $\Delta[HbD]$, indicating a complex association between UPE and tissue HD/OX; (2) UPE decreases significantly during AO but not during VO; (3) UPE increases significantly after AO; and (4) the view that ROS are the source of UPE is generally supported by the present study, although some findings remain unexplained.

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References

[1] Van Wijk RV, Van Wijk EP (2005) An introduction to human biophoton emission. Forsch Komplementarmed Klass Naturheilkd 12(2):77–83

[2] Boveris A, Cadinas E, Chance B (1980) Low level chemiluminescence of the lipoxygenase reaction. Photobiochem Photobiophys 1(3):175–182

[3] Slawinski J (1988) Luminescence research and its relation to ultraweak cell radiation. Experientia 44(7):559–571

[4] Hodgson EK, Fridovich I (1974) The production of superoxide radical during the decomposition of potassium peroxochromate(V). Biochemistry 13(18):3811–3815

[5] Boveris A, Cadenas E, Reiter R et al (1980) Organ chemiluminescence: noninvasive assay for oxidative radical reactions. Proc Natl Acad Sci U S A 77(1):347–351

[6] Sauermann G, Mei WP, Hoppe U et al (1999) Ultraweak photon emission of human skin in vivo: in fl uence of topically applied antioxidants on human skin. Methods Enzymol 300:419–428

[7] Edwards R, Ibson MC, Jessel-Kenyon J et al (1989) Light emission from the human body. Complement Ther Med 3(2):16–19

[8] Kobayashi M, Takeda M, Ito K et al (1999) Two-dimensional photon counting imaging and spatiotemporal characterization of ultraweak photon emission from a rat's brain in vivo. J Neurosci Methods 93(2):163–168

[9] Kobayashi M, Takeda M, Sato T et al (1999) In vivo imaging of spontaneous ultraweak photon emission from a rat's brain correlated with cerebral energy metabolism and oxidative stress. Neurosci Res 34(2):103–113

[10] Van Wijk EP, Van Wijk RV (2005) Multi-site recording and spectral analysis of spontaneous photon emission from human body. Forsch Komplementarmed Klass Naturheilkd 12(2): 96–106

[11] Yang J-M, Lee C, Yi S-H et al (2004) Biophoton emission and blood flow in the human hand. J Int Soc Life Inform Sci 22(2):344–348

[12] Muehlemann T, Haensse D, Wolf M (2008) Wireless miniaturized in-vivo near infrared imaging. Opt Express 16(14):10323–10330

[13] Yan Y, Popp FA, Sigrist S et al (2005) Further analysis of delayed luminescence of plants. J Photochem Photobiol B 78(3):235–244

[14] Van Wijk R, Kobayashi M, Van Wijk EP (2006) Anatomic characterization of human ultra-weak photon emission with a moveable photomultiplier and CCD imaging. J Photochem Photobiol B 83(1):69–76

[15] Rastogi A, Pospisil P (2010) Ultra-weak photon emission as a non-invasive tool for monitoring of oxidative processes in the epidermal cells of human skin: comparative study on the dorsal and the palm side of the hand. Skin Res Technol 16(3):365–370

[16] Delpy DT, Cope M, van der Zee P et al (1988) Estimation of optical pathlength through tissue from direct time of flight measurement. Phys Med Biol 33(12):1433–1442

[17] Duncan A, Meek JH, Clemence M et al (1995) Optical pathlength measurements on adult head, calf and forearm and the head of the newborn infant using phase resolved optical spectroscopy. Phys Med Biol 40(2):295–304

[18] Van Beekvelt MC, Colier WN, Wevers RA et al (2001) Performance of near-infrared spectroscopy in measuring local O2 consumption and blood flow in skeletal muscle. J Appl Physiol 90(2):511–519

[19] Nioka S, Kime R, Sunar U et al (2006) A novel method to measure regional muscle blood flow continuously using NIRS kinetics information. Dyn Med 5:5

[20] Schreiber SJ, Megow D, Raupach A et al (1995) Age-related changes of oxygen free radical production in the rat brain slice after hypoxia: on-line measurement using enhanced chemiluminescence. Brain Res 703(1–2):227–230

[21] Guzy RD, Schumacker PT (2006) Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. Exp Physiol 91(5):807–819

[22] Clanton TL (2007) Hypoxia-induced reactive oxygen species formation in skeletal muscle. J Appl Physiol 102(6):2379–2388

[23] Jeroudi MO, Hartley CJ, Bolli R (1994) Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. Am J Cardiol 73(6):2B–7B

[24] Nakamura K, Hiramatsu M (2005) Ultra-weak photon emission from human hand: influence of temperature and oxygen concentration on emission. J Photochem Photobiol B 80(2):156–160