EPR studies of coal oximetric probes in laser irradiated human melanotic SK cells

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New type of oximetric probes for determination of optimal photodynamic therapy (PDT) conditions was examined by the use of electron paramagnetic resonance spectroscopy (EPR) at X-band (9.3 GHz). It is known that paramagnetic centers of coals [1] and carbonized coal samples [2] actively interact with oxygen. The aim of this work was to find coal samples which reveal EPR lines strongly susceptible for the presence of oxygen molecules O₂ in the cells environment. Excitation of oxygen molecules from triplet (S =1) to singlet (S = 0) state was studied in human melanotic SK cell culture irradiated by laser 662 nm and 500 mW.

As oximetric probes were tested the group of medium-ranked coal carbonized at different temperatures: 400, 500, 600 and 800 °C. EPR spectra of coal probes in control cell culture were measured. The changes in the EPR spectra after laser irradiation of tumor cells were observed.

EPR measurements were performed by spectrometer with modulation of magnetic field 100 kHz. Microwave frequency was recorded. For the first derivative spectra we obtained: amplitude (A), integral intensity (I), linewidth (ΔBpp), and g factor. g-Value was calculated from resonance condition. Influence of microwave power on EPR spectra of coal samples was analysed. Microwave powers in the range 0.7-70 mW were applied. Changes of amplitudes and linewidths with increasing of microwave power were obtained.

Only a very weak EPR lines were measured for coal carbonized at 800 °C, so this sample was rejected in our studies. Condensation process of aromatic rings may be responsible for quenching of paramagnetic centers in these large aromatic structures.

Broad EPR lines were recorded for all the studied samples. Concentrations of paramagnetic centers ~10¹⁹ spin/g characterize coals carbonized at 400, 500 and 600 °C. g-Value of paramagnetic centers in the tested samples was 2.0033. Decrease of concentration of paramagnetic centers with increasing of heating temperature was observed. Microwave saturation measurements indicate that size of aromatic units increases for higher temperature of heating. Strong interactions of unpaired electrons delocalized at large aromatic structures with paramagnetic oxygen molecules O₂ exist in coal probes. Amplitudes and integral intensities decrease with increasing of oxygen content in the sample environment. Formation of quasi-chemical bonds between oxygen molecule and unpaired electron of coal is responsible for quenching of EPR signals of the probes.

It was observed that the best oximetric probe for the analysed melanotic tumor SK cells is coal carbonized at 600 °C. Application of this oximetric probe proved strong formation of singlet oxygen in laser irradiated cells. Excitation of oxygen O₂ from paramagnetic ground triplet to diamagnetic singlet state is accompanied by increase of amplitude and integral intensity of EPR line of coal heated at 600 °C.
Microscopic observation of cell culture confirmed EPR data. Cell cultures of the studied control and irradiated samples are compared in Figure 1. Decrease of number of tumor cells in the culture was observed after laser irradiation.

Fig. 1. Control cell culture and irradiated cells for 1 day after laser treatment.

References