



*“BioTechNan - the programme of interdisciplinary cross-institutional post gradual studies KNOW
in the field of Biotechnology and Nanotechnology”*

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Rozprawa doktorska pt. **„Zbadanie aktywności biologicznej różnych układów zimnej plazmy
atmosferycznej”**

Streszczenie w języku angielskim

„Biological activity of Cold Atmospheric Pressure Plasma”

The cold atmospheric pressure plasma (CAPP) is generated following the usage of high voltage to discharge gas, leading to their ionization. The achieved non-thermodynamic equilibrium state, where the temperature of generated electrons reached several times higher than that of ionized molecules with large mass, resulting in the temperature of cold plasma plume not exceeding 50 °C. Besides the ionized molecules of discharge gas, the cold plasma remains a rich source of several representatives of reactive oxygen and nitrogen species (ROS and RNS) in the gas phase, such as $^1\text{O}_2$, O_3 , H_2O_2 , O_2^\bullet , OH^\bullet , ONOO^- , NO_2^- , N_2^+ . The interaction between the cold plasma plume and liquids or solids leads to further participation of ROS and RNS in cascade reactions, forming reactive individuals with longer lifetime and significant chemical as well as biological activity.

In the presented PhD thesis, a novel CAPP system was utilized to conduct interdisciplinary research studies to assess the biological activity towards human breast cancer cell lines (MCF-7, MDA-MB-231) and non-cancerous cell line (MCF-10A) as well as pathogenic bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Serratia marcescens*, *Enterobacter cloacae*). The CAPP system called plasma pencil was used for the activation of two cultural media- DMEM and Opti-MEM, and subsequently used for the indirect treatment of human breast cancer cell lines. The investigated *in vitro* models in the second case were directly exposed to CAPP plume, while in the third case, during mixed treatment, the cells were directly irradiated with plasma pencil and then incubated in plasma-activated culture media. In order to assess the impact of indirect, direct and mixed CAPP treatment of breast cancer cell lines on cell viability, motility and apoptosis induction, biological tests were performed in particular test MTT, scratch test and Annexin V and Propidium Iodide



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cell staining. To determine the presence of ROS and RNS in the gas phase of CAPP discharge, the Optical Emission Spectroscopy (OES) technique was used. The colourimetric-based methods were utilized to determine the concentration of NO_2^- , NO_3^- , NH_4^+ , H_2O_2 , in culture media after plasma pencil treatment. Moreover, the impact of admixture of fetal bovine serum and higher contribution of nutrients in culture media on the obtained biological activity of plasma activated media along with the generation of ROS and RNS was studied.

The novel system of plasma pencil, together with the proposed construction of plasma brush which generates five plasma plumes, were optimized with a multivariate optimization route based on statistical tools dedicated to an effective degradation of antibiotics from water solutions. The main purpose of optimization studies was to obtain the operational discharge parameters with the highest efficacy of antibiotic degradation. The so-optimized systems of plasma pencil and plasma brush were used in a continuously-flowing manner for the irradiation of water solutions of Ofloxacin (OFX), Doxycycline (DXC), Ampicillin (AMP), Chloramphenicol (ChRP) with the concentration of 35 mg dm^3 , as well as the mixture of these four antibiotics with the content of each analyte equal 35 or 10 mg dm^3 . The degradation efficacy of the proposed plasma pencil and plasma brush systems was assessed using High-Performance Liquid Chromatography with Diode-Array Detection, HPLC-DAD and Ultra-Performance Liquid Chromatography with Mass Spectrometry, UPLC-MS. The changes in Total Organic Carbon (TOC) content and Total Nitrogen (TN) in pharmaceutical solutions after CAPP irradiation were measured with a multi N/C 3100 analyzer. The possibility of reducing the multiple drug resistance in pathogenic bacteria was examined with a standard disc-diffusion assay. Due to the degradation of antibiotics from water solutions subsequently, with the reduction of antibacterial activity towards *Escherichia coli*, *Bacillus subtilis*, *Serratia marcescens*, and *Enterobacter cloacae* were observed after treatment with plasma pencil and plasma brush. The observed biological activity of CAPP systems was linked with their involvement in ROS and RNS generation in antibiotic solutions. Post-plasma liquids were studied in terms of pH changes, and electrical conductivity along with colourimetric techniques for concentration determination of ions NO_2^- , NO_3^- as well as molecules H_2O_2 and total ROS contribution.