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Summary of the dissertation submitted for the degree of Doctor in Biocybernetics and Biomedical Engineering

Title: **The influence of electroporation on selected anti-tumor agents applied in human breast cancer cells**

Currently used anti-cancer therapies often turn out to be ineffective or toxic to healthy cells because of numerous side effects. Moreover, intrinsic or acquired resistance of tumor cells to drugs is a major factor in the failure of chemotherapy. Therefore, it is necessary to design and develop new strategies for the treatment of tumors. Research in this field is mainly focused on search for more effective treatments, innovative and less toxic drugs, and methods for their selective delivery.

The aim of this study was to investigate *in vitro* the possibility of application of electroporation to enhance drug's uptake in a model of multidrug-sensitive and resistant human breast cancer cells. The study evaluated the potential of electropermeabilization method for improving the efficiency of chemotherapy and photodynamic therapy.

Scientific hypotheses:

- The application of electroporation in breast cancer cells, sensitive and resistant to standard chemotherapy, can be an alternative method for anti-tumor agents delivery and increased cytotoxic effect.
- The use of electroporation will enable reduction of the drug dose and exposure time in the breast cancer cells, without loss of efficiency.

The study was performed on the model of two human breast adenocarcinoma cell lines: MCF-7/WT – the wild type line and MCF-7/DX – the line resistant to doxorubicin. Additionally, Chinese hamster ovary cells (CHO-WTT) were used as a model for transport studies of electroporation due to very low expression of endogenous ion channels. Electroporation was used to enhance photodynamic reaction based on the action of two compounds: Photofrin® (Ph, clinically used photosensitizer) and the cyanine IR-775 - commercial photosensitive compound that has not been used in photodynamic therapy.

Our own preliminary studies indicated that IR-775 may have a potential in the therapy. Bleomycin, which is a cytostatic antibiotic that causes damage to the DNA strand in cancer cells, was used as a cytostatic drug in the studies of electrochemotherapy.

The project assessed the effectiveness of a transport of the photosensitive compounds and the cytostatic drug into the cancer cells and investigated the cellular responses induced by electroporation-assisted photodynamic reaction and electrochemotherapy. The influence of the electric field and the concentration level of the anti-cancer agents on the intracellular processes were assessed. In both cell lines changes in cellular morphology and mechanisms of drug resistance (the expression of transport proteins) were visualized. Fluorescent properties of the compounds enabled the assessment of their incorporation into the cells. Cell death and cellular responses induced by electroporation and the activity of tested compounds were also evaluated. Cellular responses included changes of membrane integrity, the activity of enzymes involved in metabolism, lysosomal activity and the cell ability to proteins synthesis.

The study revealed that the selected parameters of the electric field alone (without any anticancer agent) had no significant cytotoxic effect on the cellular functions and no significant morphological changes induced by electroporation alone were observed. Also the selected parameters of photodynamic reaction or chemotherapy *in vitro,* without electroporation, had no significant cytotoxic effects on the cellular functions. On the other hand, electroporation enabled the creation of new ways for intracellular molecular transport, enhancing delivery of propidium iodide, IR-775 and Photofrin. A combination of electroporation with chemotherapy or photodynamic therapy *in vitro* effectively induced cell death. It had also a cytotoxic effect on the investigated parameters of cellular functions. Moreover, the proposed methods influenced the selected components of the antioxidant system (the expression of GST) and a system of multidrug resistance. The applied treatment was effective for both MCF-7/WT (sensitive) and MCF-7/DX (resistant) cell lines.

In conclusion, the combination of photodynamic reaction or chemotherapy with electroporation increased the cytotoxic effect of the treatment *in vitro* in human breast cancer cells. The application of electric field for cell membrane permeabilization could enable application of new compounds and reduction of side effects via reduction of drug dose and the exposure time, in comparison to the standard parameters used in anticancer therapy. Moreover, the selectivity of the treatment can also be improved, as permeabilization occurs only near the area of the delivery of pulses. Therefore, electroporation, which is already used for chemotherapy enhancement, may be also considered as an attractive system

for photosensitizers delivery. The detailed examination could be continued at the *in vivo* level, giving the possibility of planning and development of new anticancer strategies with particular emphasis on cells resistant to current treatments.