Under the influence of the electric field action, changes occur in the structure of a cell membrane. Formation of transient pores allows penetration of molecules into cells, which transport under normal conditions is limited or even impossible. The technique utilizing this unusual phenomenon is called electroporation (EP) and it is currently widely applied in biotechnology. However, a truly innovative approach is its use in anticancer therapy as electrochemotherapy (ECT), when cytostatic drugs are administered simultaneously with the application of the external pulsed electric field. Increasing the permeability of cell membranes via electroporation can have a significant impact on the improvement of drugs' effectiveness, and hence – on the reduction of drug dosage and limitation of side effects associated with therapy. Unfortunately, due to the primary and acquired resistance of pancreatic cancer cells, the effects of treatment are still not satisfactory. Moreover, current regimens include only two drugs with relatively high toxicity - bleomycin and cisplatin, which cannot be used in all patients. This indicates a clear need to introduce new therapeutic substances into the ECT protocols and to increase the effectiveness and selectivity of this method for malignant, drug-resistant cancers. This can be achieved, inter alia, by sensitizing cell membranes to the pulsed electric field. Accordingly, the project is designed to:

- **study and compare the efficacy of EP using calcium as an alternative to standardized drugs**
- **sensitize cancer's cellular membranes to ECT using low toxic antioxidant and low toxic prooxidative iron compounds.**

Our working hypothesis here, based on preliminary data, is that preincubation of drug-resistant cancer cells with low-toxic antioxidant (catechin) and prooxidative iron compounds (ferric citrate and EDTA iron(III) sodium salt) may enhance lipid membrane oxidation and thus the efficacy of applied pulsed electric field and finally electrochemotherapy.

The presented project combines both - theoretical and experimental approach. The first stage of the project assumes the development of a computer model of cellular membranes, which will be subjected to simulations of the interaction with external electric field and with the tested compounds – catechin, calcium and iron ions. This will allow us to deepen the knowledge about the mechanisms taking place in the membranes at the atomic level, which will then be verified in macroscale under experimental conditions. The creation of an appropriate research model opens up huge possibilities for simulating experiments. It will allow to test interaction with a virtually unlimited number of factors and to optimize the parameters prior to *in vitro* experiments. This may result in a significant reduction in the costs of running biological experiments. Developed and verified the model of EP and ECT can be subsequently utilized by research teams from around the world. For *in vitro* experiments, we will use two cell lines of pancreatic cancer with varying degrees of drug-resistance and a cell line of normal cells as a control. The study includes two models of cell culture – cells classically kept in monolayers and miniaturized 3D culture in which cells form the aggregates. The use of this model will allow spatial relations between cells to be taken into account without the need to use an animal model. Additionally, the developed protocol of aggregated cells electroporated in gel could be transferred to other research units. In the presented project, the evaluation of the cellular response to EP and ECT focuses primarily on evaluation of multidrug resistance proteins, enzymes involved in detoxification and the type of cell death promoted by therapy. Investigating these mechanisms may increase the understanding of the phenomenon of drug resistance and the processes that occur in cancer cells. The obtained results hereby can contribute to the design of new, effective therapeutic strategies.