The aim of the proposed project is to develop a new approach based on microfluidic Lab-on-a-Chip system and pancreatic islets' 3D models ("pseudoislets") to perform high-throughput studies aimed at functional analyses of pancreatic islets under physiological and pathological conditions.

In our research, we will create a three-dimensional model of a cellular aggregate ("pseudoislets") consisting of the two most important cell types for diabetes mellitus type 2: ß cells (INS-1E) that secrete insulin and α cells (α-TC1-6) responsible for secretion of glucagon. Such a model will be more similar to the conditions prevailing in the human body than the monolayer or three-dimensional static culture. We will start our research by developing a microfluidic system with geometry of structures that will allow aggregation of cell culture. This system will be made of biocompatible materials suitable for cell culture that will provide observation and analysis of results. In addition, the microsystem used in the research will enable analysis of changes in one "pseudoislet" of a specific, definite size. Pancreatic islets are composed of many types of cells present in a strictly defined ratio and location. The second step will be to select the appropriate cellular composition to reflect these proportions. Confirmation of a well-chosen ratio of cells will be examined by performing immunofluorescence staining and observation of obtained structures in three-dimensional space. The next stage of research will be to choose the flow conditions in the microsystem to obtain a long-term culture with high vitability and proliferation level. The influence of flow conditions will be examined on the basis of microscopic assessment of changes in cell morphology and tests such as: Alamar Blue, BrdU, propidium iodide/calcein AM.

The next and final stage of our work will be to estimate of insulin secretion in the developed microfluidic system depending on various flow conditions and glucose stimulation. As insulin secretion is a complex, two-stage process with the dynamic nature of biomolecule release, research should be made using a method that allows measuring a large number of samples over time. Nowadays, most of the studies are based on a single measurement of insulin, and the results obtained are only the averaged value of insulin concentration in time interval that has elapsed since introduce of the factor. Based on previous studies and measurement of the insulin secretion by immunological ELISA assay we will select the method suitable for continuous measurement of insulin secretion level. At this stage, we will attempt to apply detection using Surface Plasmon Resonance in the developed microfluidic system. Thanks to use this method, we will be able to determine the small amounts of secreted insulin in samples with a volume of several dozen µl in real time. Moreover, this method gives the opportunity to measure the time of association and dissociation of molecules on the surface of the detector and thus determine the affinity and kinetics of the interactions.

The team takes this subject of research, due to the possibility of a deeper understanding of the glucose dependent insulin secretion. The idea of using a microfluidic system is associated with the necessity to create a model of pancreatic islet reflecting the conditions prevailing in the body. In the proposed project, we will attempt to create a microfluidic system that will provide ideal conditions for long-term "pseudoislets" culture, observation of the single islet function and measurement secreted insulin level. We believe that the developed model could be a universal model for conducting further research.