1. Research project objectives/Research hypothesis

Studies on pathophysiology of diseases contribute to the development of better treatment methods. Such studies may be conducted in a laboratory vessel (*in vitro*) using models applying human cells and simulating conditions in the body. One such model is a blood vessel model with endothelial cells (EC), which in the human body cover the inner surface of the heart, arteries and veins with a single layer. The EC control transport of different molecules between the blood and the tissues, they secrete substances necessary for proper functioning of the human body. The EC are easily activated and respond quickly to stimuli. Studying these responses contributes to a better understanding of the development of many diseases, including diabetes, which affects more than 400 million people and leads to numerous complications, including blindness, heart attack and stroke. These complications are caused by prolonged high level of sugar in blood, which promotes the formation of free radicals and dysfunction of EC. *In vitro* studies demonstrate a greater effect of variable sugar concentration than constant high sugar level on functioning of EC. However, the results of clinical trials of the effect of the blood sugar variability on the increased risk of diabetic complications are not conclusive. The above-mentioned *in vitro* studies were performed under conditions differing from those existing in the human body. Within the blood vessels, the force caused by the blood flow acts on EC, which significantly influence cells' shape and functions. We developed a method of seeding and controlled culturing of the human EC in a special device (bioreactor) on the inner surface of the low diameter semipermeable plastic pipes (capillaries), which mimic the shape of blood vessels. In the bioreactor, as in veins, the force caused by the flowing culture medium, which replaces blood, acts upon EC.

The aim of the project is to develop a model to study the effect of variable sugar concentration on functioning of EC. The developed model will reproduce the impact of the force acting on EC, which is caused by the flow of culture medium. In order to better mimic the structure of the blood vessel, this model will be refined by embedding smooth muscle cells (SMC) on the outer surface of capillaries.

2. Research project methodology

So far, the applicant has developed and verified in laboratory conditions methods for: (1) manufacturing and preparation of a capillary bioreactor for the deposition of cells, (2) introduction of EC into the bioreactor and seeding cells on the inner surface of the capillaries using own rotary device, (3) optimization of culturing conditions for the long-term cell culture. The developed and verified methods of seeding EC and their culturing in the bioreactor ensure a uniform coverage of the inner surfaces of capillaries by cells. We preliminary tested the method of simultaneous insertion and seeding of EC and SMC on the inner and the outer surface of capillaries, respectively. This method requires improving to achieve full coverage of capillaries by SMC. The possibility of culturing EC and SMC separately in laboratory dishes in culture media containing glucose in constant or variable concentration was confirmed. Within the project we will develop and integrate the bioreactor with an automatic flow control and glucose concentration control system, which will make it possible to simulate normal, constantly high and variable level of sugar concentration. The developed model will be used to determine the degree to which constant level and variability of the sugar concentration influences functions and survival of EC. For this purpose, a series of experiments will be conducted to analyze substances produced by or stimulating EC, which illustrate the condition and processes occurring in these cells. In addition, it is planned to investigate the effectiveness of selected drugs mitigating the unfavorable influence of sugar on EC.

3. Expected impact of the research project on the development of science

Determining the effect of glucose concentration variability on processes leading to the development of the diabetic angiopathy will help to define the optimal treatment for diabetes and may lead to a significant slowdown in the development of its complications, contributing to the prolongation of life expectancy among persons with diabetes. The results of the project may provide a basis for explaining mechanisms of the development of complications of diabetes and, in the long run, contribute to the development of effective methods of treating them. **The model developed in this project may be used in the future to continue investigations on diabetes and its late complications. Moreover, the developed model may be easily adjusted to study influence of the level and variability of other substances on EC, and thus, making it possible to investigate other diseases affecting EC (e.g. atherosclerosis, stroke, chronic kidney failure, tumor growth, cancer metastasis, venous thrombosis) and to study effects of using drugs.**