THE POPULAR SCIENCE PROJECT SUMMARY

Currently, civilization diseases are a major challenge for the diagnostics and pharmacology fields. One of the most serious disease is broadly defined cancer. The cardiovascular disease, such as atherosclerosis, are also a serious clinical problem beside the cancer. In both cases, it is important to determine risk factors, diagnosis of disease at an early stage and the use of a suitably treatment. Therefore, searching of a advanced and precise diagnostic tools is reasonable.

It turns out, that the detection of disorders of the coagulation system may help to predict the development of both atherosclerosis and cancer diseases (Nash GF., Et al., *The Lancet Oncology* 2001). Thrombin, as a key component of the coagulation system, favors the development of atherosclerotic plaque and has influence on rising risk of thrombo-embolic incidents which are complications of atherosclerosis. It can also stimulate the proliferation of vascular endothelial cells and tumor cells (Nash GF., Et al., *The Lancet Oncology* 2001). What is more, thrombin also promotes intravascular clotting and tumor-cell – platelet interactions (Nierodzik M. L. et al., J. *Clin. Invest.* 1991; Tsopanoglou NE., Et al., *J. Biol. Chem.* 1999). Moreover, it can cause that the endothelial cells are more sensitive to the action of Vascular Endothelial Growth Factor (VEGF) (Möhle R. et al., *Proc. Natl. Acad. Sci.*, 1997), It leads with increasing of endothelial cells mitosis and cell migration. The occurrence of Venous Thromboembolism (VTE) among cancer patients is further evidence of the correlations between dysfunction of coagulation system and cancer diseases (Mege D. et al., *Thrombosis Research* 2016). One of the identified risk factors for VTE among cancer patients is hypercoagulable state, which results from increased thrombin generation. The von Willebrand factor is an another important component of the coagulation system which is released by activated endothelial cells, promoting the development of thrombosis and tumor metastasis (A. Bauer, T. et al., *Thrombosis and Hemostasis* 2016).

Therefore, developing a method to study the condition of the coagulation system is important to assess the state of cancer development and cardiovascular pathologies. The proposed solution is a biosensor which detection is based on the usage of aptamers. Aptamers are single-stranded synthetic oligonucleotides which undoubted advantage is the high specificity and selectivity for a particular ligands (Ruscito A. et al., *Frontiers in Chemistry* 2016). The small size of oligonucleotides and their single-stranded structure allows them to change conformation, which ensures strict connecting to an epitope of the target molecule. Hence, aptamers are gaining interest in the therapeutic and diagnostic fields. Aptamers, as a tool for the development of biosensors, are competing for antibody widely used in the methods of qualitative and quantitative analysis and anticancer therapies. The aptamers can specifically bind to e.g.: toxins, antibiotics, molecular markers or heavy metals (Ruscito A. et al., *Frontiers in Chemistry*, 2016). Examples of aptamers directed to thrombin and vWF which are planned to use are shown in ***Table 1***.

Table 1. Examples of aptamers sequences directed to thrombin and vWF (Trapaidze A. et al., *Biosensors and Bioelectronics* 2016; Sundaram P. et al., *European Journal of Pharmaceutical Science* 2013; Huang R.-H. et al., *Structure* 2009).

APTAMER	LIGAND	SEQUENCE
HD1	Thrombin	5’-GGTTGGTGTGGTTGG-3’
HD22	Thrombin	5’-AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3’
ARC1779	vWF	5’-GCGUGCAGUGCCUUCGGCCGTGCGGTGCCUCCGUCACGC-3’
ARC1772	vWF	5’-GGCGTGCAGTGCCTTCGGCCGTGCGGTGCCTCCGTCACGCC-3’

The studying of aptamer-protein interactions is necessary to implementation the imperative aim of the project. The assessment of impacts can be carried out by Capillary Electrophoresis usage. This technique allows to obtain information about the dissociation constant K_d of an aptamer-ligand complex and the rate constants of the association (k_{on}) and dissociation (k_{off}) processes. The obtained values will allow to assess the suitability of the selected aptamers as the capture and detection biomolecules in the context of the created biosensor. The superparamagnetic microparticles labeled with a well-profiled mixture of fluorescent dyes are the base of the biosensor. Microparticles are used in the Luminex technology which allows to detection of several analytes in one sample in a single measurement cycle (multiplex technology). The microparticles surface modification by appending tosyl groups leads to surface activation and it allows the microparticles surface coating by aptamers at subsequent steps of the project. The tosylation process will be controlled by using the Raman spectroscopy and Flow Cytometry (FCM) methods. The biosensor will be used to carry out analysis of mouse plasma samples (Balb/C mice: orthotopic breast cancer line 4T1 model, ApoE/LDLR$^{-/-}$ mice: advanced atherosclerosis model) by Luminex multiplex technology. The control method will be Calibrated Automated Thrombogram (CAT) for thrombin detection and ELISA Kit for vWF detection.