1. OPIS PROJEKTU DOKTORSKIEGO (4000 znaków max., łącznie z celami i planem pracy, do umieszczenia na stronie internetowej Szkoły)

Tytuł projektu: Role of purinergic signaling in the processes of regeneration, repair and remodeling with endothelial cells

1.1. Cele projektu

The main aim – disclosing the potential of purinergic signaling in regeneration, repair and remodeling processes, will be achieved by below listed particular goals:

A. Screening of endothelial cells (ECs) *in vitro* cultures (2D and 3D models) in terms of purinergic signaling influence on their proliferation, migration, and tube formation
B. Assessing the *in vitro* effects of adenylate kinase immobilized on nanomaterials on the cytophysiology of endothelial cells
C. Developing the nanobiocatalytic tool maintaining the nucleotide balance with potential therapeutic activity towards endothelial disturbances

1.2. Ogólna charakterystyka projektu

Processes of regeneration, repair and remodeling are important in the pathology and physiology of different cells, including endothelium. The pivotal regulatory role in these processes is ascribed to the extracellular matrix and signaling within it [1]. Therefore, dynamic molecular changes in the tissue microenvironment determine the modifications of cell biology reflected either individually or collectively. **Cytophysiological properties of endothelial cells (ECs) underpin several events and phenomena, such as endothelial injury, inflammation, vasculogenesis, and angiogenesis** [2].

Purinergic signaling components (nucleotides, nucleosides, receptors, enzymes) contribute significantly to the modulation of physio- and pathological processes such as cell proliferation, differentiation, inflammation, immune response, and apoptosis in various human cells and tissues [3-5]. Extracellular purine nucleotides, mainly ATP and ADP, through the activation of P2 receptors in the vasculature and in blood platelets, participate in the initiation and spreading of aggregation and inflammation [6]. On the other hand, adenosine is accepted as an anti-inflammatory and pro-proliferatory compound, that can be also responsible for the proliferation and migration of endothelial cells [7]. It is worth noting that purinergic signaling system must be precisely regulated to maintain nucleotide homeostasis within tissues [5]. Thus, **factors that maintain the appropriate balance between ecto-purines deserve special scientific attention**.

Our previous results proved that the key component of purinergic signaling is the set of membrane-bound and soluble enzymes metabolizing nucleotides and nucleosides in the

extracellular environment [8]. The main role of those enzymes includes regulating the duration of action of the receptor agonists, as well as providing the products as ligands for the purinoreceptors. The main purine-hydrolyzing enzymes are: ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), alkaline phosphatases (APs), ecto-nucleotide pyrophosphatases/phosphodiesterases (E-NPPs), ecto-5'-nucleotidase (E-5'-NT), and adenylate kinase (AK) [9]. **Adenylate kinase is one of the few enzymes involved in the metabolism of nucleotides that does not catalyze their hydrolysis, but regulates their concentration through the phosphate moiety transfer**. This unique property allows the level of nucleotides to be continuously and precisely maintained in non-pathological ranges [10], without affecting the expression of proteins related to purinergic signaling.

We assume that purinergic compounds could be exploited to control the processes of proliferation, migration and tube formation of endothelial cells. As the pleotropic effects of purines seem to be dependent on cellular context and microenvironment, the novel purine-based approach will be thoroughly studied *in vitro* in an appropriate model, reflecting the 3D interactions within tissue. The results will be beneficial from the therapeutic point of view as many clinical problems are connected with disturbances in ECs cytophysiology. One of them is restenosis, which is an overgrowth of endothelium in a blood vessel occuring after stent implantation. The implantation procedure always induces damage to the vessel endothelium, which is manifested by intense, long-term inflammation. **Our hypothesis assumes that adenylate kinase immobilized on biocompatible carbonaceous nanomaterial can effectively regulate the concentration of ecto-nucleotides in the ECs microenvironment**. It can therefore be considered as a potential nanobiocatalytic therapeutics with antithrombotic, anti-inflammatory and anti-restenotic effects.

1.3. Plan pracy

A. Optimization of endothelial cells (ECs) culture in three-dimensional models
B. Molecular and biochemical characterization of purinergic pathway components in ECs
C. Assessment of purinergic signaling influence on ECs' proliferation, migration, and tube formation
D. Immobilization of adenylate kinase and evaluation of its capability to control ecto-purines balance

1.4. Literatura *(max. 10 pozycji/sugestia lektury dla kandydatów)*

1. Evans CE, Iruela-Arispe ML, Zhao YY (2021) Mechanisms of Endothelial Regeneration and Vascular Repair and Their Application to Regenerative Medicine. Am J Pathol 191(1): 52-65.
2. Krüger-Genge A, Blocki A, Franke RP, Jung F (2019) Vascular Endothelial Cell Biology: An Update. Int J Mol Sci 20(18): 4411.
3. Burnstock, G (2007) Purine and pyrimidine receptors. Cell Mol Life Sci 64: 1471.

4. Burnstock, G (2011) Introductory overview of purinergic signaling. Front. Biosci. (Elite Ed), 3(3): 896–900.
5. Vultaggio-Poma V, Falzoni S, Salvi G, Giuliani AL, Di Virgilio F (2022) Signalling by extracellular nucleotides in health and disease. Biochim Biophys Acta Mol Cell Res 1869(5): 119237.
6. Koupenova M, Ravid K (2018) Biology of Platelet Purinergic Receptors and Implications for Platelet Heterogeneity. Front Pharmacol 9: 37.
7. Galgaro BC, Beckenkamp LR, van den M Nunnenkamp M, Korb VG, Naasani LIS, Roszek K, Wink MR (2021) The adenosinergic pathway in mesenchymal stem cell fate and functions. Med Res Rev 41(4): 2316-2349.
8. Roszek K, Wujak M (2018) How to influence the mesenchymal stem cells fate? Emerging role of ectoenzymes metabolizing nucleotides. J Cell Physiol, 234(1): 320-334.
9. Yegutkin GG (2014) Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. Crit Rev Biochem Mol Biol, 49(6): 473–497.
10. Hetmann A, Wujak M, Bolibok P, Zięba W, Wiśniewski M, Roszek K (2018) Novel biocatalytic systems for maintaining the nucleotide balance based on adenylate kinase immobilized on carbon nanostructures. Mater Sci Eng C, 88: 130-139.

1.5. Wymagana wstępna wiedza i umiejętności kandydata/tki na doktoranta/kę

- knowledge on biochemistry, cytophysiology and *in vitro* cell culture
- understanding of biochemical and molecular biology techniques, and at least basic practical experience in using them
- analytical thinking
- open to challenging tasks and creative
- hard-working person, eager to learn

1.6. Oczekiwany rozwój wiedzy i umiejętności kandydata/tki na doktoranta/kę

- innovative thinking
- ability to plan and organize laboratory work
- skilled in novel scientific techniques
- ability to solve research problems
- ready to work in international research group