The liver is a well-vascularized organ, through which rich in nutrients blood from the digestive system flows in a volume of up to one liter per minute. Nutrients, including lipoprotein molecules, are distributed through a network of small capillaries, called liver sinusoids. The first layer of cells in the liver sinusoid is the layer of endothelium. Liver sinusoidal endothelial cells (LSECs) constitute a single thin layer of cells separating the lumen of a blood vessel from the remaining liver cells, mainly hepatocytes. There exist a colloquial expression, that the liver is a blood filter. The term, however very generalized, finds its justification in the morphology of LSECs. These cells have fenestrations – transcellular pores – which gathered into groups build sieve plates for molecules suspended in blood plasma. Fenestrae have sizes ranging from 50-300 nanometers. Passive transport through fenestrae to the liver includes only those lipoproteins which are smaller than the size of a pore. Large particles, such as chylomicrons, remain in the plasma, where they are reduced by enzymes. Therefore, fenestrae in LSECs constitute an active barrier, allowing for a controlled flow of lipoproteins to and from the liver. In liver pathology, resulting from i.e. obesity, inflammation, or alcohol abuse, the number of fenestrae drops, and thus the filtering capacity of this organ is disturbed. This leads to hyperlipoproteinaemia and, consequently, can lead to atherosclerosis and cardiovascular disease. In summary, disturbances in the porosity of LSECs may have consequences in abnormal gastrointestinal and cardiovascular systems.

Despite the enormous function of fenestrae in LSECs, the knowledge about the processes regulating changes in their number and size remains rudimentary. This is related to the limitations of the research techniques of these structures. As fenestrae are smaller than the wavelength of visible light (400 - 900 nanometers) they cannot be seen under the optical microscope. To study them, it was necessary to use electron microscopy. This, however, requires complicated preparation protocols. This has prevented the study of dynamic changes of these structures. For nearly 50 years since the discovery of fenestrae, the speed of processes associated with the changes in the number and size of fenestrae remained speculative. With the development of atomic force microscopy (AFM) imaging methodology, new, so far out of reach, methods of investigating the porosity of LSEC cells appeared. In 2017, using AFM, we showed fenestrae in living cells for the first time. Currently the methodology developed by the principal investigator of this project remains the only one in which it is possible to track the dynamic changes of fenestrae.

The current literature research, including ours which is based on AFM imaging, indicate the huge role of the cytoskeleton in the structure of fenestrae. **The aim of this project is to understand the role of the cytoskeleton in fenestrae formation and maintenance, as well as the influence of vasoprotective mediators on the porosity of live primary, freshly isolated murine LSECs in vitro. The experiments will be conducted in three models of defenestration in LSECs.**

AFM microscopy is already used to assess the condition of endothelial cells using a different marker - nanomechanics (elasticity, stiffness). As changes in nanomechanics are also associated with changes in the structure of the cytoskeleton of endothelium and allow the detection of early changes in the condition of these cells, we will include the study of this marker in the conducted experiments. There are currently no literature reports on changes in LSECs' nanomechanics. In this project, we will provide data on alteration in LSECs' nanomechanics in response to various factors. What is more, we will perform the first correlation of changes in the porosity and nanomechanical properties of LSECs cultured on soft hydrogel-based substrates. We hypothesize that the porosity of LSECs is regulated by vasoprotective mediators (e.g. NO, PGI_2, VEGF), acting indirectly on the cytoskeleton.

The research proposed in this project is clearly innovative. First of all, they will be the first studies on dynamic changes in the porosity and nanomechanics in response to drugs. In addition, confirmation of the contribution of selected elements of the cytoskeleton, forming fenestrae, will allow for the design of the therapy allowing restoring the porosity of dysfunctional liver. If the change in the mechanical properties of the substrate is sufficient to increase cell porosity, it will provide pioneering data on the *in vivo* regulation of fenestration. Furthermore, combined knowledge about alterations in the porosity and nanomechanics of LSECs and the influence of endothelial mediators on fenestrae may be used as a platform to study the toxicity and reversibility of drug action at a sophisticated level at the nanoscale. Finally, the research will provide information on the phenotypic changes in the cytoskeleton in real time. The interactions between cytoskeletal elements in living cells, both intact and exposed to pharmacological tools, can be translated into other cell types. This will allow you to learn about the dynamics of the LSEC cytoskeleton and to understand the mechanisms regulating fenestrae, which is of direct importance for the treatment of liver diseases.