Near Infrared (NIR) radiation (780 nm  $\div$  2500 nm) is currently used for therapeutic purposes, e.g. in physiotherapy, dentistry, rheumatology and dermatology. It plays a particularly important role as an effective method of pain relief and regeneration. Our previous research has shown that it affects the coagulation system, causing reversible changes in their functional activity over time. In addition, it stabilizes the membranes of erythrocytes, increasing their resistance to destructive factors, and has antioxidant properties. In recent experiments performed on an animal model of extracorporeal circulation using an artificial heart-lung machine, we have proved that NIR photobiomodulation (PBM) of blood can be successfully used for cytoprotective purposes. Despite the huge application potential, the practical use of this discovery is extremely difficult due to the lack of comprehensive and well-systematized knowledge regarding the regulation of the basic cellular properties of modified erythrocytes, as well as their interaction with other blood cells and endothelial cells of blood vessels. Understanding these processes is the goal of the proposed project.

The average life span of red blood cells (RBCs) under physiological conditions is about 120 days. Therefore, the content of blood products used in transfusion medicine is a mixture of both young, mature and senescent erythrocytes, which are characterized by different antioxidant properties and morphology. In particular, changes in morphology that directly translate into deformability and compressibility of erythrocytes are crucial in the proper performance of the main function of these cells, which is to ensure high efficiency of gas exchange. At the first stage of the project, a miniature device for sorting and electrochemical analysis of erythrocytes will be developed. The device will be an alternative to the commonly used method of centrifugation of cells in a density gradient, which in optimal conditions should be carried out in highly specialized centrifuges (so-called ultracentrifuges). In addition, the use of impedance methods will allow the development of equivalent models for individual cell groups, describing the electrical properties of the cell membrane and cytoplasm.

Next, we will examine the effects of photobiomodulation of erythrocytes with NIR radiation using the in vitro aging model and the aforementioned microfluidic platform. At this stage, we will perform 3D morphology imaging using digital holotomography and a whole series of biophysical and biochemical tests to assess the antioxidant status and changes in the structure of the cell membrane. The aspect of the biomechanical properties of the cell membrane will be examined using optical tweezers. At the same time, we will determine the profile of changes in parameters accompanying the eryptosis process, i.e. the so-called programmable red cell death. The last task will be to study the interaction of erythrocytes after NIR modulation with other blood cells and endothelial cells in a microfluidic model of a blood vessel.

The implementation of the project will contribute to answering many fundamental questions regarding the potential benefits and limitations related to the practical application of photobiomodulation of erythrocytes and blood using NIR radiation.