Raman4Cell2Cell: Uncovering the reciprocal interactions of leukocytes and endothelial cells by (stimulated) Raman imaging

Our body behaves like a musical orchestra with different instrumental sections that can be attributed to different systems (circulatory or lymphatic system) in the human body. Appropriate interplay and communication of all systems is very important for proper functioning of the body. With the development of civilization, the environment is changing, which can affect our orchestration in the body, causing certain disorders, named as diseases of civilization. They affect a very large percentage of the population and are now considered responsible for about 80% of premature human deaths. The development of civilization diseases is related to chronic cell inflammation in which communication between leukocytes (Leu) and endothelial cells (ECs) is crucial. These cell-cell interactions in inflammation take place not only by sending intercellular signals, but in many cases also by direct contact between these cells, and this process is called transmigration. The process is very complex and can be divided into several stages: differentiation of stem cells into selected subtypes of Leu, turning, adhesion to ECs, adhesion and migration to organs through the endothelial cell layer (Fig. 1). The main aim of this project is to explain the interactions and mutual influence of leukocytes and endothelial cells during inflammation using *in situ* Raman spectroscopy (RS) techniques.

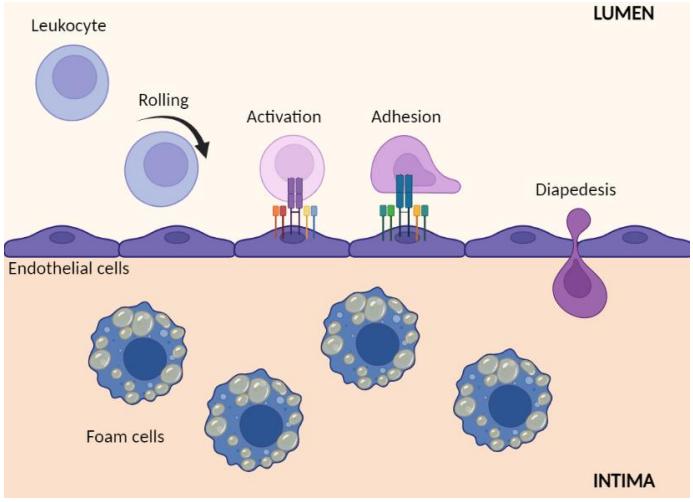

Fig. 1. Scheme of leukocyte transmigration (Created with BioRender.com).

Leu plays a key role in the immune system, both adaptive and innate immunity. In order for them to become fully functional cells capable of fighting against target cells, it is necessary to activate them in response to immune response factors. Currently, T cell activation has been proposed in CAR-T therapy, which is a highly advanced and personalized form of cancer immunotherapy. Different subpopulations of Leu are involved in different inflammatory responses, e.g. lymphocytes T in atherosclerosis, while monocytes in coronary artery disease, and in these processes their migration through endothelium to inflammatory sites is provoked. The endothelium is a single layer of cells lining the interior of vessels with many functions and tasks. Any disturbance in the proper functioning of the ECs affects the functioning of the whole blood system, as many diseases such as cancer, diabetes or atherosclerosis are closely linked to endothelial dysfunction. Moreover, inflammation-activated Leu can affect the state of the ECs, which in the longer term may lead to impaired functioning of the circulatory system. Lipids and carotenoids are particularly important substances involved in inflammatory processes and their role in these processes is well known. Nevertheless, their metabolic changes still remain unexplained.

The hypotheses posed within this project are: **i/** in vitro stimulation of inflammation in ECs models of lifestyle diseases activate selected Leuk and their activation occurs in various ways; **ii/** the presence of carotenoids in Leu cells changes the efficiency of their transmigration through ECs; **iii/** the efficiency of Leu transmigration differs in normal cells and cells exposed to exogenous fats. The hypotheses are also strongly correlated with the **stated goals**, which include: **1/** to build a Spectroscopic Atlas of Leukocytes - unique spectroscopic markers for selected Leu subtypes will be selected; **2/** to identify spectroscopic markers of Leu activation and biochemical changes in the process of adhesion/transmigration through ECs in inflammation; **3/** to determine changes in the process of Leu activation induced by inflammation in ECs; **4/** to track changes in carotenoid and lipid exchange between Leu and ECs in the transmigration process with the use of RS.

Nowadays many techniques are used to study cell-cell interactions, such as fluorescence microscopy, transmission electron microscopy, flow cytometry or mass spectrometry. However, what is worth emphasizing, these methods provide information only on global changes in the cells, such as the liquidity of cells or the presence of proteins/receptors on the cell surface. To fully understand these processes the full molecular, morphological and metabolomical information are required. **Such a composite of information can be provided using a range of *in situ* Raman techniques** (RS, stimulated Raman scattering – SRS, coherent anti-Stokes Raman spectroscopy – CARS) accompanied by other established methods (fluorescence microscopy and flow cytometry). In the project is planned a 3M Raman characterization (molecular, morphological, and metabolic) of both Leu and ECs during their communication. The ability to exchange and transport of substances (carotenoids and lipids) between Leu and ECs will be evaluated, as well as an assessment of qualitative changes in labeled lipids. Raman spectroscopy provides detailed information about the composition and allows to track changes over time in single cells.

Research on intercellular communication between Leu and ECs, including and intercellular transport, is an innovative approach that will contribute to a better understanding of the mechanism of inflammatory processes linked to selected civilization diseases. It may contribute to new treatment strategies, especially for chronic inflammatory conditions that are still challenging to treat.