In recent years more attention has been put on studies related to mitochondria to elucidate these organelles as a therapeutic target in the treatment of many human diseases. Since **mitochondria play a pivotal role** in cell function and cell death, they are also involved in the progression and pathogenesis of many diseases, e.g., cardiovascular and neurodegenerative disorders or cancer. Mitochondrial disorders are estimated to occur in about one in five thousand individuals, making it one of the most common types of human diseases. Mitochondria in endothelial cells (EC) occur in low amount and have a small contribution to energy production under normal physiological conditions but play very important regulatory roles. EC have an important physiological function in vascular homeostasis. They are also the first barrier that separates blood from deeper layers of blood vessels and extravascular tissues. Thus, they are exposed to various physiological blood components as well as challenged by pathological stimuli, which may exert harmful effects on the vascular system by stimulating of excessive generation of reactive oxygen species (ROS). The major sources of ROS are NADPH oxidase and mitochondrial respiratory chain complexes.

Directly visualizing biological structures and activities at the cellular and subcellular levels remains so far one of the most intuitive and powerful ways to study biological problems. Each organelle plays a specialized and essential role in cellular processes. Our ability to investigate biological systems at the microscopic level has been completely transformed by rapid improvements in light microscopy. Typically, the method of choice for visualization of many distinct molecular species in order to understand such complex systems as cells is fluorescence microscopy. However, the major issue in fluorescence organelle imaging is the photostability of fluorescent labels and their size is rather bulky limiting utility for imaging small molecules. Moreover, it has been shown that in some cases they can alter the physiology of the sample. An attractive alternative to fluorescence microscopy offers Raman microscopy (RM). RM belongs to a small group of non-invasive methods that do not require an additional exogenous labels to be an effective technique. Raman spectra of cells are complex since they contain information about all the molecules occurring in the sample, providing insight into chemical structure and changes taking place in the cells. When compared to fluorescence microscopy, which is regarded as a reference method, Raman imaging is considered as a label-free approach, and this is sometimes pushed as an advantage because it provides a comprehensive image of the biochemistry of the cell. Therefore, **a new approach of using Raman probes** (Rp), a labeled variant of label-free Raman imaging, was presented. It is a relatively new methodology used in subcellular research, usually allowing much better selectivity and sensitivity than label-free Raman spectroscopy. Labeled RM takes advantage of the silent region (1800 – 2800 cm-1) in the Raman spectra, where no characteristic signal from biomolecules is included in cells.

The **research hypothesis** of the project is about the usefulness of the newly developed and available Rps in tracking the processes taking place in the complex biological systems, in particular individual cell organelles in various *in vitro* models. One of the **main objectives** of this project is to search for spectral markers of endothelial and mitochondrial dysfunction at the subcellular level by using several spectroscopic techniques, including labelling. This will allow obtaining new and useful information on cell metabolism and hence to be used in the diagnosis of physiological functional changes occurring upon development of various pathological situations at the cellular level.