Endothelial inflammation studied *in situ* within functional blood vessels using Raman imaging, atomic force microscopy and imaged cytometry

The vascular endothelium forms the innermost layer of all blood and lymphatic vessels. Together with the underlying basement membrane it forms the tunica intima. This inner layer is surrounded by the *tunica media* which, depending on local mechanical and metabolic needs, predominantly consists of vascular smooth muscle cells and elastin fibres. Endothelium is a thin, unicellular layer of highly specialized cells lining the interior surface of blood and lymphatic vessels. It is not only a physical barrier between blood and tissue, but it also plays a plethora of essential functions in the organism. It regulates vascular tone, permeability of the vessel wall, smooth muscle cell proliferation and migration, trafficking of inflammatory cells between blood and underlying tissue, angiogenesis, innate and adaptive immunity organ regeneration, fibrinolysis and inflammation.

This project involves the measurement of *in situ* endothelial cells within isolated functional blood vessels. This means that after dissection of the blood vessel from the mice body, the split-open vessel wall is kept in the special medium supporting the functionality of endothelial cells. The project involves the development of comprehensive methodology including high resolution Raman imaging, Raman spectroscopy *via* Raman fiber probes, AFM imaging (topography, phase and amplitude), force distance curve measurements and biological functional assays for analysis of *in situ* endothelial cells.

Enlargement of background knowledge about influence of the inflammation state on *in situ* endothelial cell seems to be important step toward specifying the markers of inflammation state in the endothelium. The results of chemical alterations (e.g. creation of lipid bodies measured by Raman imaging), physical changes (e.g. increasing stiffness checked by AFM force distance curves measurements) or reduced amount of NO (biological assays) upon stimulation by pro-inflammatory agents will be characterized and compared. The next step of the project assumes application of combination of methods to pharmacology of endothelial cells within functional isolated blood vessel. In the selected model of inflammation, the effectiveness of endothelium-protective agents like statins or angiotensin converting enzyme inhibitors on depicting markers of inflammation will be evaluated.