Nanoscale investigation of cigarette smoke effects on human bronchial cells using atomic force microscopy

Leading cause of death in the Western world are vascular diseases like atherosclerosis, the latter is caused mostly by smoking. Although the connection between smoking and cardiac diseases have been known for a long time, the detailed effects of tobacco smoke ingredients are still unclear. This is also due to a lack of in vitro studies. Our collaborator research group developed an evaluation of an in vitro model for analysis of tobacco smoke effects on cell cultures and tissues.[1] They developed a special cigarette smoke sampling device, in which the smoke goes through from a gas-to a liquid-phase leading to an in vivo-identical nicotine concentration. With this device we can investigate the effects of smoke ingredients in vitro, but very similar to the in vivo conditions. Cell morphology and cell death analysis, high-performance liquid chromatography, mass spectrometry was used for examination of the smoke treated cell cultures and tissues to investigate the basic biological mechanisms of the active tobacco smoke.

Although a lot of information about molecular effects of smoking were gained, the effects of smoke for the cell morphology on the nanoscale has not been investigated yet. The goal of our ongoing project is to investigate membrane changes of the human bronchial cell line (HBE3-KT) on the nanoscale using atomic force microscopy (AFM) imaging techniques.

During this study we are working with an immortalized cell line (HBE3-KT), a human endothelial cell line isolated from the lung. We culture the cells and treat them for 24 hours with different concentration of tobacco smoke treated feeding media. After smoke treatment the cells are fixed and investigated using AFM. Currently we are interested in morphological changes of the cell membrane on the nanoscale. Therefore we investigate and compare differently treated cells and characterize by number, type, quality, and level of membrane damages. Our goal is to complete the information about the molecular changes with changes of cell morphology on the nanoscale which can help to understand the effects of smoke in more detail, and which can be helpful for the development of new treatments for patient with atherosclerosis. Later, the investigations will be extended to single molecule receptor characterization and localization (for a review see [2]).

During your master thesis you will do cell culturing at our partners lab following a well-established protocol, including the smoke treatment of the cells. You will prepare different samples for AFM measurement's and fluorescence pre-characterization. You will image the cells with different types of AFM techniques at varying environmental conditions. A detailed characterization on the single molecule level by using single molecule force spectroscopy as well as simultaneous topography and recognition imaging is planned with our partner. You will analyse and prepare your data in close collaboration with your supervisor. You will get a full training in all used techniques.

If this topic arouses your interest, contact us: Andreas Ebner (andreas.ebner@jku.at) and Michael Leitner (michael.leitner_1@jku.at)

Looking forward to meet you!

[1] Bernhard, David, et al. "Development and evaluation of an in vitro model for the analysis of cigarette smoke effects on cultured cells and tissues." Journal of pharmacological and toxicological methods 50.1 (2004): 45-51.

[2] Kienberger, Ferry, et al. "Molecular recognition imaging and force spectroscopy of single biomolecules." *Accounts of chemical research* 39.1 (2006): 29-36.