IRTG 914 - Advanced Methods Course – summer semester 2018

"Transmigration studies using a novel Pore Membrane Flow Chamber"

Polymorphonuclear neutrophils as important players of the innate immune system represent the first leukocytes recruited to sites of injury during an acute inflammatory response following a well-defined multistep cascade of adhesion and activation events. Extravasation of neutrophils from the blood stream into the inflamed tissue is an important step for the recruitment of neutrophils to sites of inflammation. The aim of the Advanced Method Course is to introduce the Pore Membrane Flow Chamber. This device and the state of the art technology spinning-disk confocal microscopy (SDCM) allow live cell imaging of *in vitro* neutrophil transmigration under flow conditions. The µ–Slide membrane flow chamber consists of a flow chamber which can be coated with different recombinant proteins, e.g. rmICAM-1 and rmP-selectin, a 300 nm thick membrane with 5 µm pores and a subjacent collagen gel with a chemoattractant, e.g. CXCL1 or fMLP. During the course we will perfuse isolated murine neutrophils through the coated flow chamber with 1 dyne/cm^2 shear stress and analyze neutrophil adhesion and transmigration using SDCM. The acquired 3D images (movies) will be analyzed using Slidebook 6.0.8 Software (3i, USA).

Speaker/Supervisor: Melanie Salvermoser (AG Walzog)

Literature:

1. Salvermoser M, Pick R, Weckbach LT, et al. Myosin 1f is specifically required for neutrophil migration in 3D environments during acute inflammation. *Blood.* 2018.

2. https://ibidi.com/blog/transmigration-and-transport-studies-through-a-porous-glass-membrane-introducing-ibidis-slide-membrane-ibipore-flow-n13