4-D in vivo imaging of podocytes in a zebrafish injury model

***Florian Siegerist*[1], *Weibin Zhou*[2], *Karlhans Endlich*[1], *Nicole Endlich*[1]**

[1]*Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany*
[2]*Departments of Pediatrics and Communicable Diseases and Human Genetics, Ann Arbor, Michigan, USA*

In the past, it was postulated that podocytes are able to migrate along the glomerular basement membrane (GBM), especially in podocytopathies. Recently, we have shown that podocytes in healthy zebrafish larvae neither migrate along the GBM nor show motility of their processes (Endlich et al. 2014), remaining static over up to 23 h. In this study, we wanted to clarify whether podocytes exhibit a migratory phenotype after the induction of injury.

To study this *in vivo,* we used the NTR/MTZ zebrafish model expressing mCherry and nitroreductase (NTR) which converts the prodrug metronidazole (MTZ) to a cytotoxin specifically in podocytes (Zhou et al. 2012).

For *in vivo* observation by 2-photon microscopy (2-PM) this zebrafish strain was crossed with the translucent zebrafish *Casper* and named *Cherry*. The application of MTZ (5 mM) to *Cherry* larvae *(*3 days post fertilization) for 20 h induced apoptosis which was demonstrated by TUNEL assay. We found decreased levels of nephrin and podocin mRNA measured by qRT-PCR. Moreover, we observed foot process effacement of the remaining podocytes and a widespread denudation of the GBM shown by immunohistochemistry and electron microscopy.

Since podocin was downregulated after MTZ application, we crossed *Cherry* with *ET* (Endlich et al. 2014) resulting in a zebrafish strain (*Chet)* with podocytes expressing additionally eGFP. Currently, we can follow the dynamics of podocytes of up to 31 larvae simultaneously over 24 h by 2-PM. In a time-dependent 3-D reconstruction (4-D) of z-stacks of the glomerulus, we found that the remaining podocytes retracted their major processes and that Bowman's space dilated significantly. However, no *podocyte walking* was observed during 24 h in zebrafish larvae (n=61).

Taken together, we show that podocytes do not migrate along naked GBM in our zebrafish injury model during the early time period of 24 h.