APPLICATION OF MULTI-PHOTON FLUORESCENCE MICROSCOPY TO MONITOR 3D THROMBUS FORMATION AT STENOTIC SEGMENTS IN THE MOUSE CAROTID ARTERY

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Background
Exacerbated thrombus formation after atherosclerotic plaque rupture is the main cause of acute myocardial infarction and stroke and is driven by interplay between the thrombogenic contents of ruptured plaques and the local shear environment. The pro-aggregatory effects of the shear environment at stenotic plaques remain poorly characterized. Since intraluminal growth of atherosclerotic plaques is typically confined to large vessels, the use of animal thrombosis models of the macro circulation is warranted.

Aim
We developed a 3-dimensional reconstruction method applied to resonant scanning mode multi-photon microscopy to investigate real-time thrombus formation during acute arterial stenosis in the carotid artery in mice.

Results
High speed acquisition (8000 lines/sec) was used to monitor thrombus formation with sub-micrometer resolution in the common carotid artery of mice. Local stenosis was induced by indentation with a 27G needle. The stenosis initiated exacerbated thrombus formation in the stenosis outflow region. The angular difference between the optical plane and the carotid artery was exploited to obtain interplane z-distances from standard time lapse acquisitions. Based on that, an off-line reconstruction method was developed to obtain 3-dimensional information on intra-aggregate domains of fibrin(-gen), P-selectin and platelets. Complementary in vitro studies in microfluidic flow chambers with eccentric stenotic segments were used to characterize the mechanism underlying stenosis dependent thrombus formation. The unique shear conditions provoked by atherosclerotic plaque geometries spatially confined thrombus formation due to local alterations in platelet reactivity, increased plasma vWF adhesion and endothelial cell activation.

Conclusion
This study highlights the potential use of multi-photon fluorescence microscopy to image platelet activation/aggregation and coagulation factors in 3D in the macro circulation of mice, in particular during acute arterial stenosis.

Keywords Multi-photon microscopy, arterial thrombosis, microfluidics