Molecular ultrasound imaging

Summary

The endothelium is the inner lining of blood vessel walls with direct contact to the blood circulation. It plays a major role in all stages of atherosclerosis, from its initiation to plaque rupture and thrombosis. Endothelial dysfunction, triggered by abnormal shear stress, local inflammation, and chemokine release is followed by i) the upregulation of surface adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and selectins, and ii) the translocation of platelet endothelial cell adhesion molecule (PECAM)-1 and junctional adhesion molecule (JAM)-A, which facilitate the transmigration of inflammatory cells from circulation into the arterial wall.

Oscillatory arterial blood pressure and hyperlipidemia, or injured endothelium following revascularization procedures predispose patients to develop atherosclerotic lesions and neointima, respectively. During revascularization procedures (**Chapter 3** and **Chapter 4**), such as balloon angioplasty with/without stent implantation or endarterectomy, the endothelial layer is severely injured and partially or fully removed. Throughout the process of endothelial regeneration, the blood vessel is vulnerable to thrombus formation and restenosis. Recovering endothelial cells expose biomarkers on the luminal surface, which can be used as potential target for imaging and early diagnosis. Similarly, oscillatory arterial blood pressure and hyperlipidemia cause endothelial activation with luminal exposure of biological markers (**Chapter 5**).

In this study, the main aim was to establish a disease-specific molecular imaging vascular contrast agent that can withstand the physiological flow and shear stress conditions in major arteries. Therefore, two clinically related murine models of endothelial dysfunction mimicking endothelial denudation (**Chapter 3** and **Chapter 4**) or oscillatory arterial blood pressure (**Chapter 5**) were used: the wire-injury (WI) and the partial-ligation (PL), respectively. The arteries were screened by immunohistology for particular biological markers. As contrast agent, polymer-based air-filled microbubbles (MB) with the diameter of 1-2 μm were employed.

MB functionalization was performed by conjugation of specific antibodies to the MB surface. Fluorescent loading of the MB shell enabled the bimodal detection of the particles using both two-photon laser scanning microscopy (TPLSM) and molecular ultrasound (US). Clinical imaging modalities, such as US imaging, lack resolution for studying the interaction of microbubbles with the vascular wall. Therefore, TPLSM was applied as a deep-tissue imaging modality for the characterization of surface marker expression and binding kinetics of rhodamine-loaded MB as a preclinical validation step.

Firstly, using TPLSM imaging on TNF-α stimulated explanted murine carotid arteries in an *ex vivo* flow chamber system, the shear stress resistance of ICAM-1-targeted MB (MB_{ICAM-1}) was characterized at shear rates matching and exceeding physiological parameters (**Chapter 2**). ICAM-1 was chosen as target for endothelial inflammation after immunohistological screening of TNF-α-stimulated carotids for several biological markers. TPLSM results were further used as validation of *ex vivo* and *in vivo* molecular US imaging employing MB_{ICAM-1} in the same experimental animal model.

Next (**Chapter 4**), the endothelial recovery after arterial denudation was monitored by employing VCAM-1-targeted MB (MB_{VCAM-1}). VCAM-1 is expressed immediately after endothelial denudation on the medial smooth muscle cells. During early vascular recovery, it is expressed on both smooth muscle cells and regenerating endothelium, but it disappears from the endothelial layer after its structural recovery. This makes it the perfect molecular marker to track endothelial healing. Using *in vivo* TPLSM, single MB bound to the vascular lumen were imaged and quantified showing specific retention. Translation into molecular US confirmed injury-specific MB retention and accurate assessment of the endothelial regeneration state in correlation with immunohistology and TPLSM imaging of the luminal surface.

Lastly, transient endothelial activation under acute blood flow variations was investigated *in vivo* by employing JAM-A-targeted MB (MB_{JAM-A}) for molecular US imaging of carotid arteries subjected to PL (**Chapter 5**). JAM-A is an inter-endothelial marker which redistributes to the luminal surface upon inflammatory

stimuli and endothelial activation. MB_{JAM-A} bound specifically to JAM-A on activated endothelium and are able to identify the location of areas with endothelial dysfunction upon acute changes in blood flow. Our data indicate that not only flow reduction due to vessel obstruction in the partially ligated artery, but also acute flow increase due to compensatory blood flow redistribution in the contralateral carotid, induce luminal exposure of JAM-A. This illustrates the high sensitivity of this marker for assessing acute vascular vulnerability and remodeling.

In conclusion, this thesis provides new insights into molecular imaging of atherosclerosis and clinically relevant associated biological processes, specifically neointima formation and flow-induced endothelial activation. After further refinements, these imaging methods may become important tools in both the scientific and the clinical environment.