Dual-modality imaging of rhodamine-loaded microbubbles targeted to ICAM-1 under controlled shear stresses

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Endothelial dysfunction and inflammation play a critical role in the progression of atherosclerosis. The overexpression of surface adhesion molecules during the early stages of atherosclerosis is characteristic in major vessels such as the carotid artery. However, imaging of carotid atherosclerosis in the early stage is challenging due to high flow and shear forces. Therefore, it is critical for preclinical optimization to understand the binding kinetics and behaviour of contrast agents on inflamed endothelium under different shear stresses at the cellular level.

Polymer-based microbubbles (MB) based on poly-butylcyanoacrylate (PBCA) with an average size of ~2 µm remain in the vascular compartment and target primarily the endothelium. They can be used for a broad range of biomedical applications such as chemical conjugation, drug loading, and noninvasive detection via contrast-enhanced ultrasound (CEUS). Therefore MB are highly suitable for molecular imaging of overexpressed surface receptors on angiogenic and/or inflamed endothelium.

The shell of MB can be loaded with optical contrast agents such as rhodamine, which enables dual-modal imaging with optical imaging modalities. Two-Photon Laser Scanning Microscopy (TPLSM) is a deep-tissue optical imaging method for the direct visualization of bound targeted MB on fresh explanted or in vivo carotids. The submicron resolution of TPLSM offers the possibility to visualize single MB and consequently quantify the total number of MB bound to the endothelium in the field of view.

Here, we show that rhodamine-loaded PBCA-MB can be used as a bimodal contrast agent for the detection of inflammation markers via TPLSM using ICAM-1 as our model target. ICAM-1-targeted MB bind specifically to TNF-α stimulated HUVEC and ex vivo murine carotid arteries at flow rates 0.25 ml/min - 1 ml/min and shear rates up to 100 dyn/cm². Furthermore, we show that after wire injury of the carotid artery and high fat diet in ApoE-/- mice, ICAM-1 targeted MB bind specifically to the inflamed endothelium can be imaged using in vivo TPLSM. Our data provide the basis for the development of clinical contrast agents in disease-burdened areas with high shear and flow rates.