Visualization of atherosclerotic lesions in carotid arteries of mice using Two-Photon Laser Scanning Microscopy

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Atherosclerosis involves structural and cellular changes in the vessel wall of large arteries. Microscopic imaging of such structures requires good optical sectioning, large penetration depth, and subcellular resolution. Two-Photon Laser Scanning Microscopy (TPLSM) combines these properties, enabling imaging of large intact arteries. In the present study we demonstrate that TPLSM combined with specific fluorescent markers can be used to study development of atherosclerotic lesions in carotid arteries of ApoE-/- mice (14-16 or 20-22 weeks of age, fed a western diet for a period of 6 or 12 weeks, resp.). Isolated carotid arteries, including the bifurcation, were mounted in a home-built perfusion chamber, pressurized (up to 80mmHg transmural pressure), and imaged using a water dipping objective (60X, NA=1.0). Structures of the arterial wall were visualized using specific fluorescent markers for cell nuclei (SYTO41), macrophages/monocytes (CD11b/PE), elastin (eosin), and collagen (CNA35/OG488, a novel collagen type I, III, and IV marker).

Preliminary results show that atherosclerotic lesion development can be detected. Earlier stages of lesion development were present at lesion-prone sites in arteries, such as monocytes adhering to the vessel wall and intimal clustering of macrophages. Furthermore, a subtle increase of intimal collagen content was detectable in lesion areas as well as in non-lesion areas. Matured lesions contained large numbers of CD11b-positive cells, and showed alterations in elastin and collagen structure and content.

These results confirm TPLSM as a powerful tool to investigate the development of atherosclerosis in large intact arteries.