

IMAGING OF COLLAGEN AND ASSOCIATED STRUCTURES IN ATHEROGENESIS IN MURINE CAROTID ARTERIES USING TWO-PHOTON MICROSCOPY

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Two-Photon Microscopy (TPM) combines optical sectioning, penetration depth, subcellular resolution, and reduced photodamage. We used TPM to study atherogenesis in carotid arteries of 2 murine models. We also visualized collagen exposure *in vivo* after mechanical damage in mouse carotid arteries.

In an (*ex vivo*) model ApoE^{-/-} mice (15 or 21 weeks) were fed a western diet from week 8 on, with age matched C57Bl6/J mice on normal chow as controls. Isolated viable carotids were mounted, pressurized, and labeled in a perfusion chamber, and imaged using TPM. General cell content (Syto41) and relation between collagen (CNA35/OG488) and inflammatory cells (anti-CD11b/PE) were visualized as marker for plaque instability. Control vessels had intact endothelium, no adhering cells and no intimal collagen labeling. In young ApoE^{-/-} mice, inflammatory cells adhered to endothelium of common carotid, accompanied by intimal collagen labeling in absence of lesions. In plaques located in bifurcation of ApoE^{-/-} mice, inflammatory cell density increased with age (15 to 20 cells/ (100 μm)³, p<0.05) and plaque progression (12 to 28 cells/ (100 μm)³, p<0.01). In fibrous caps alone, inflammatory cell-collagen contact increased with age (p<0.05). In plaques as a whole, neither aging nor plaque progression altered cell-collagen contact.

In an *in vivo* model we concentrated on continuity of collagen availability in lesions induced by collar placement in ApoE^{-/-} (15 weeks old, collar after 2 weeks western diet) carotid arteries. Three or 6 weeks after collar placement, CNA35 linked to Quantum Dots (CNA35-QDs) was injected via the femoral vein; untargeted streptavidin coated QDs served as control. Carotid arteries were excised 2 hours later and imaged as described above. Clear hotspots of labeled collagen were visible, indicative for enhanced permeability for relatively large QDs at specific, presumably vulnerable, sites within the plaque. This is in contrast to CNA-OG, which labeled entire plaques.

Finally, we injected CNA35-QDs in C57BL6/J mice *in vivo*. One of their carotid arteries was prepared free and imaged *in vivo* using the TPM triggered to heart rate and respiration. No collagen labeling was observed. In contrast, after inducing mechanical damage to the carotid wall by forceps pinching, immediate collagen labeling of the subendothelial basal membrane was observed, demonstrating the potential of classical TPM systems for *in vivo* molecular imaging.