

## Two-photon imaging of liposome uptake by intact, inflamed mouse carotid arteries.

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### Introduction

Atherosclerosis is a chronic inflammatory disease characterized by an increased uptake of leukocytes and lipoproteins by the vessel wall, mediated not only by an increased expression of adhesion molecules on the endothelium, but also by increased vessel wall permeability. We determine the 3D distribution of conventional and sterically stabilized ('stealth') liposomes in inflamed carotid arteries (CA) of ApoE<sup>-/-</sup> mice.

### Methods

Inflammation was induced by two-day wire occlusion of the right CA of ApoE<sup>-/-</sup> mice. Conventional and stealth liposomes, containing the fluorescent probe Rhodamine Lissamine (RL), were injected into the left jugular vein. After 24 hours of circulation blood samples were taken to test for the presence of liposomes in the circulation. Both CA were taken out and mounted in a home-built perfusion chamber. A transmural pressure of 80mmHg was applied to mimic physiological conditions. The vessels were incubated with the SYTO13 for 30 minutes. This stain visualizes the nuclei of endothelial cells, smooth muscle cells and fibroblasts [1]. The arteries were mounted in a Nikon E600FN upright microscope with 40x (NA = 0.8) and 60x (NA = 1.0) water-dipping lenses. A BioRad Radiance 2100 multi-photon system was used, with excitation at 800nm. The emitted fluorescence was spectrally separated to detect auto fluorescence (450/80nm) and fluorescence from SYTO13 (528/50nm) and RL (590/70nm).

### Results

After 24 hours the stealth liposomes were still circulating in the blood, whereas the conventional liposomes were almost completely degraded. The right (damaged) CA, but not the left (control) CA, showed a disrupted endothelial layer, replaced by various layers of different cell type. The stealth liposomes have migrated into the vessel wall and their 3D distribution in mainly the luminal layer can be visualized. They are mainly found in the cell cytoplasm.

### Discussion

The results provide information on the passive uptake of liposomes. In the future specific targeting could increase the homing efficiency of liposomes. The visualization of stealth liposomes with TPLSM appears an adequate method for imaging of inflammation. This will allow molecular imaging of the initial inflammatory phase of atherosclerosis *in vivo*.

[1] M.A.M.J. van Zandvoort; W. Engels; K. Douma; L. Beckers; M.G.A. oudeEgbrink; M. Daemen, and D.W. Slaaf, "Two-photon microscopy for imaging of (atherosclerotic) vascular wall: a proof of concept study," *J. Vasc. Res.*, **49(1)**, accepted for publication (2004).