Enhanced Imaging of Atherosclerotic Plaques in an ApoE-deficient Mouse Model using Liposomal Nanotechnology

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Introduction. Atherosclerosis is the major cause of mortality in the western world today. The main objective for the use of nanotechnology was to address prospects of imaging atherosclerotic (AS) plaques, which lead to clinical endpoints, with appropriate biomarkers (BMs) coupled to liposomal nanoparticles (NP). Today various BMs are known to be critically involved in the pathophysiologic scenario of AS-plaques. The BM-targeted NPs were investigated towards their potency to characterize critical scenarios within early and advanced AS-plaque lesions applying an AS-mouse model.

Methods. Aortas of wt and ApoE-deficient mice, fed a high fat diet, were dissected and first stained with fluorescence-labelled BMs. *Ex vivo* imaging was performed using confocal laser-scanning microscopy (CLSM). Second, the native BMs were coupled to fluorescence-labelled Stealth®-liposomes to enhance the imaging performance. For all BM-conjugates Western Blot (WB) analysis was used to assess structural changes. Modified native gel electrophoresis was used for the characterization of the BM-targeted, fluorescence-labelled Stealth®-liposomes.

Results. Successful labelling of the selected BMs with fluorescence dyes was achieved. According to WB analysis no critical structural changes occurred. CLSM imaging showed that all chosen BMs bind to the AS-plaque but not to the lesser injured aortic surface. Successful coupling of native BMs to reactive PEGylated lipids exposed on the liposomal surface of fluorescence-labelled NPs was shown by native gel electrophoresis and WB analysis. Compared to the plaque-staining using only fluorescence-labelled BMs, the BM targeted NP conjugates showed marginal to strong signal enhancement in CLSM imaging, depending on the selected BM.

Conclusion. Results by now suggest a promising role of the applied BM-targeted NPs for enhanced AS-imaging *in vivo* and their potential use for new targeted therapeutic strategies in cardiovascular medicine.