Towards optical and MR imaging of vulnerable atherosclerotic plaques using bimodal quantum dots

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Introduction

Molecular imaging is a highly promising method to discriminate between stable and vulnerable plaques, which is not possible using conventional imaging techniques. For molecular imaging, target-specific probes are required. Annexin A5 (AnxA5) binds phosphatidylserine (PS) that is exposed on the surface of dying cells, activated macrophages and platelets residing in vulnerable plaques[1-3]. AnxA5 has been used successfully to image vulnerable plaques in animals and in patients by nuclear imaging[3-5]. A new bimodal nanoparticle was designed to image these plaques by Magnetic Resonance Imaging (MRI), allowing high resolution visualization. Furthermore, the nanoparticle allows subcellular localization in intact tissues by Two-Photon Laser Scanning Microscopy (TPLSM).

Methods

The nanoparticle consists of a quantum dot (QD), a brightly fluorescent, non-bleachable dye[6], conjugated to AnxA5 (figure A). Lysine-wedges, each containing eight Gadolinium-DTPA complexes (figure B) are added to the QD. This nanoparticle was first tested *in vitro* on apoptotic (dying) Jurkat cells and whole-blood clots by both TPLSM and 1.5T MRI (inversion recovery TSE sequence with an in-plane resolution of 208x208 μm^2 and TI/TE/TR 546/13/1580 ms and 610/13/1500 ms for cells and clots, respectively). In addition, a wire-injured murine carotid artery (in which both cell death in the vessel wall and thrombus formation were induced) and a control carotid artery were mounted into a perfusion chamber and labeled *ex vivo* (intraluminally) with the nanoparticle and examined by both TPLSM and 7T MRI (multi-slice, spin-echo sequence with TE/TR 10.2/800 ms and an in-plane resolution of 89x89 μm^2).

Results

Relaxivity (r_1) of the nanoparticle in solution was calculated to be 3000-4500 $mM^{-1}s^{-1}$ per particle at 1.5T and room temperature. TPLSM demonstrated specific AnxA5-binding of the nanoparticle to apoptotic Jurkat cells. MR images of these cells showed a 34-fold increase in signal intensity compared to unlabeled cells, confirming bimodality of the nanoparticle. TPLSM also showed AnxA5-specific binding of the nanoparticle to activated platelets in whole-blood clots. MRI of whole-blood clots showed a signal intensity increase of nearly 50% compared to control clots. In the damaged carotid artery, uptake of the nanoparticle was evident with TPLSM in the tunica intima (layer from the lumen to the internal elastic lamina, containing endothelial cells) as well as the tunica media (layer between the internal and external elastic laminae, containing smooth muscle cells) of the vascular wall (figure C). Moreover, nanoparticle uptake resulted in an MR signal intensity increase in the damaged carotid artery compared to the undamaged artery (figure D).

Discussion

This bimodal nanoparticle enables imaging of apoptotic cell samples and whole-blood clots in fluorescence microscopy and MRI. Furthermore, it was shown that this nanoparticle can be used to label vascular structures *ex vivo*. It is a promising new nanoparticle for atherosclerosis research *in vivo*, as is currently under investigation.

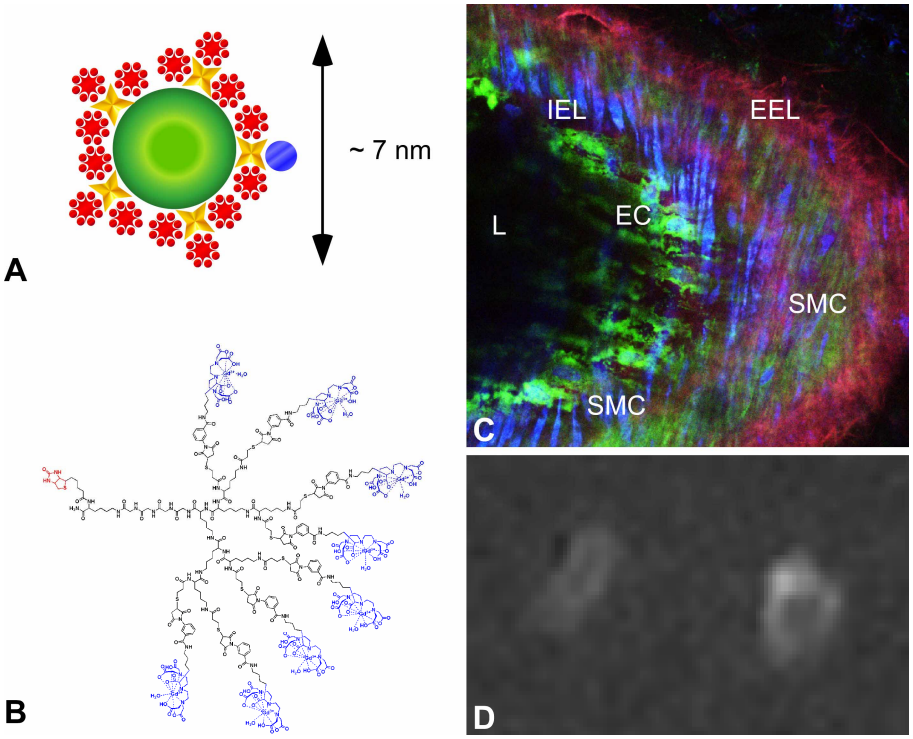

Figure A: schematic representation of the nanoparticle. Green, QD; yellow, streptavidin; blue, AnxA5; red, lysine wedge containing GD-DTPA.
B: molecular structure of the lysine wedge containing eight Gd-DTPA complexes.
C: TPLSM image of a damaged murine carotid artery, *ex vivo* labeled with the nanoparticle (green), eosin, labeling elastic lamina (red) and syto41, labeling cell nuclei (blue). L, lumen; IEL, internal elastic lamina; EEL, external elastic lamina; EC, endothelial cells, SMC, smooth muscle cells. Uptake of the nanoparticle is visible in the ECs in the tunica intima, and in the SMCs in the tunica media.
D: transversal 7T MR image of two *ex vivo* carotid arteries: control (undamaged, left) and damaged (right). The damaged artery shows a higher signal intensity.

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