Inorganic core HDL applied for macrophage imaging

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Introduction

Cardiovascular disease is one of the most prevalent causes of mortality in the world and adverse cardiovascular events are most commonly linked to rupture of atherosclerotic plaques. Macrophages have been identified as intimately linked to the progression of atherosclerosis[1] and thus the ability to non-invasively image this cell type would be advantageous. High density lipoprotein (HDL) is naturally targeted to atherosclerotic plaques and we have previously modified the phospholipid coating of HDL to act as an MRI contrast agent.[2] In this report we will discuss a new generation of HDL-based contrast agent where the cholesterol ester/triglyceride core has also been modified via substitution for inorganic nanoparticles, such as gold for CT/MRI, iron oxide for MRI or quantum dots for MRI/optical imaging (**Figure 1**). These particles allow the multimodal imaging of macrophages. We present here the synthesis and characterization of the particles and, as an example, the results of incubation of the gold HDL with macrophages analyzed by MRI, CT, confocal microscopy and TEM.

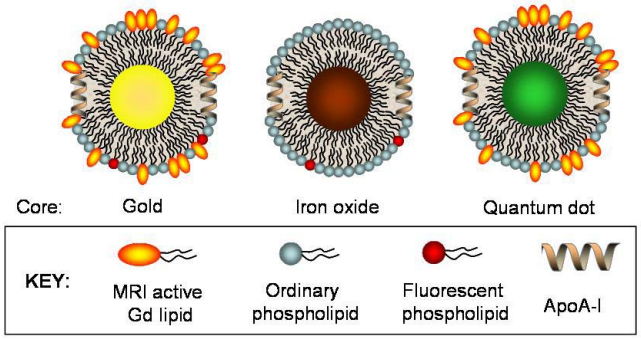

Figure 1 Schematic of the paramagnetic, fluorescent, inorganic core, HDL-based nanoparticles

Materials and Methods

The synthesis was achieved for each particle in a similar fashion, where the phospholipids and inorganic nanoparticles, *i.e.* gold, iron oxide, quantum dots, were co-dissolved in a chloroform-methanol solvent mixture. This solution was slowly added to hot buffer, forming the encapsulated particles. ApoA-I was included in the phospholipid layer via incubation. Purification was achieved via centrifugation, filtration and washing. Lipid only coated particles were also formed as controls. These nanoparticles were characterized via TEM, ICP-MS, relaxometry, CT imaging, phosphorous and protein quantification.

The gold HDL was incubated with J774A.1 macrophages for ½ 1, 2, 4, 7 and 12 hours. HeLa cells were also incubated with the agent to verify specificity, for 2 and 4 hrs. Relevant commercially available contrast agents, Omnipaque and Magnevist were used as controls. The cells were collected as a loose pellet and analyzed using different imaging methods. T1-weighted imaging and T1-mapping were done on a 9.4 T MRI scanner. and CT images of the cell pellets were acquired on a Siemens Somatom Emotion 6. The cellular localization of the gold HDL was established using confocal laser scanning microscopy and TEM. For the former method, the nuclei were stained with DAPI and for the latter method the cells were fixed in glutaraldehyde and stained with osmium tetraoxide and uranyl acetate.

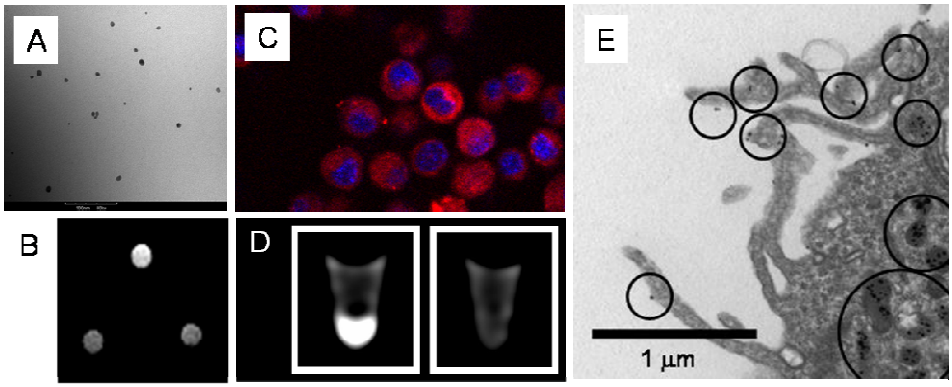

Figure 2 Gold HDL: A) TEM of the particles, B) T1 weighted images of cell pellets incubated with (clockwise from top) AuHDL, Gd-DTPA and media only, C) Confocal microscopy, D) CT image of cells incubated with Au HDL (l) and media only (r), E) TEM image of particles binding and being taken up by macrophages. Circles highlight the gold particles.

Results and Discussion

The synthesis and purification of the particles resulted in individually dispersed particles (**Figure 2A**). The relaxivities of the gold particle were found to be r1=13.1 $(mMs)^{-1}$ and r2=16.8 $(mMs)^{-1}$, comparable to values found for quantum dots (another inorganic particle) in a similar phospholipid coating.[3] Protein analysis showed that the apoA-I readily adsorbed onto the particle.

T1-weighted MRI images of the cell pellets indicated significant uptake of the gold HDL as compared with controls (**Figure 2B**). This was confirmed with confocal microscopy, CT imaging and TEM, as shown in **Figure 2C**, where gold particles can be seen binding to the cell and being internalized. CT imaging of the cell pellets revealed very great differences in attenuation (ca. 250 HU) after only 1 hr of incubation. Similar incubation experiments using iron oxide or quantum dot core HDL indicated that they also are avidly taken up by macrophage cells.

Conclusions

We have successfully synthesized novel nanocrystal containing HDL-based contrast agents which are readily taken up by macrophages. These particles have great potential as a multimodal contrast agent platform and will be soon be applied for the investigation of atherosclerosis *in vivo*.

References

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