Lipid-coated silica nanoparticles; a contrast agent platform for multimodality molecular imaging

R. Koole[1], M. M. van Schooneveld[1], J. Hilhorst[1], K. Castermans[2], G. J. Strijkers[3], C. de Mello Donegá[1], D. Vanmaekelbergh[1], A. W. Griffioen[2], K. Nicolay[3], Z. A. Fayad[4], A. Meijerink[1], and W. J. Mulder[4]

[1]Condensed Matter & Interfaces, Utrecht University, Utrecht, Netherlands, [2]Department of Pathology, Maastricht University, Maastricht, Netherlands, [3]Biomedical NMR, Eindhoven University of Technology, Eindhoven, Netherlands, [4]Department of Radiology, Mount Sinai School of Medicine, New York, New York, United States

Introduction: Nanoparticles hold great promise for biomedical applications and in the field of diagnostic imaging nanoparticles have shown very valuable for targeted molecular imaging purposes. For molecular MRI iron oxide nanoparticles, perfluorocarbon nanoparticles and liposomes have been employed extensively (1). Recently, semiconductor nanocrystals, also known as quantum dots (QDs), have been recognized as an optical contrast agent due to their outstanding fluorescent properties (2). A major advantage in the use of nanoparticles for biomedical purposes is the option of integrating multiple properties in a single device. The possibility for surface modification creates additional flexibility for a large range of applications and has resulted in the synthesis of nanoparticles that can be employed for different imaging modalities, so-called multimodal contrast agents (3). Here we report on a novel multimodal contrast agent that consists of a silica nanoparticle that contains a quantum dot in the centre. The silica nanoparticle is coated with paramagnetic and pegylated lipids to allow MRI and to improve the bio-applicability. In addition we have used the PEG-lipids to introduce target-specificity and we show the specific uptake of this novel contrast agent by endothelial cells (HUVEC) *in vitro* using multiple imaging techniques.

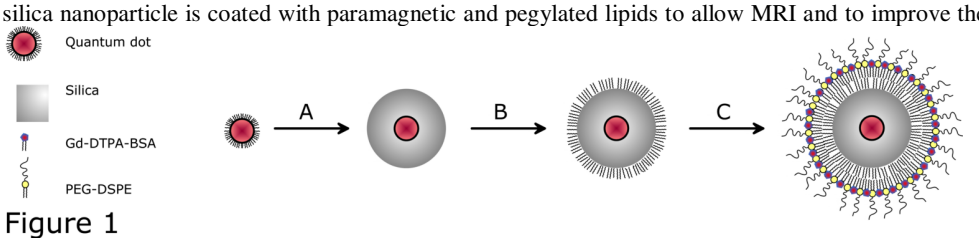

Figure 1

Material and Methods: QDs were incorporated in the centre of highly monodisperse silica particles (Figure 1, step A). After incorporation, QDs retained a high quantum efficiency (QE) of 35%. The silica particles were made hydrophobic by an octadecanol coating (Figure 1, step B). For functionalization, improved biocompatibility, and introduction of paramagnetic properties the hydrophobic silica particles were subsequently coated with both Gd-DTPA-BSA (50%) pegylated lipids (PEG-DSPE, 40%), and pegylated lipids that distally carried a maleimide group (Mal-PEG-DSPE, 10%) for conjugation (Figure 1, step C). The synthesis procedure is schematically summarized in Figure 1.

The nanoparticles were characterized in terms of their size with TEM, optical properties with spectrophotometry, and magnetic properties using a 60 MHz Bruker Minispec.

To introduce target-specific properties to this contrast agent we conjugated αvβ3-specific RGD-peptides covalently to the distal ends of maleimide functionalized PEG lipids incorporated in the outer lipid layer of the particle. HUVEC were incubated with either αvβ3-specific particles, bare particles, or were left untreated. The association of the particles with the cells was assessed using fluorescence microscopy, optical imaging, and quantitative and T1-weighted MRI.

Results and Discussion: The mean size of the quantum dot containing silica particles was determined to be 43 nm ± 5 nm as determined with TEM (Figure 2A). The emission and absorption spectra, typically for QDs, are shown in Figure 2B. The emission maximum lies at 630 nm, which is favorable for *in vivo* fluorescence imaging because of the lower autofluorescence levels of tissue for longer wavelength excitation and the better penetration depth into tissue of light with this wavelength. The longitudinal relaxation rates R1 of a dilution series were determined at 60 MHz, and Gd contents of these samples were determined by ICP-MS. From these data the longitudinal ionic relaxivity r1 was calculated to be 14.4 $mM^{-1}s^{-1}$ (Figure 1C). Taking into consideration that a particle carries approximately 13,300 lipid molecules, half of which are Gd-DTPA-BSA, the relaxivity per particle was estimated to be 96,000 $mM^{-1}s^{-1}$.

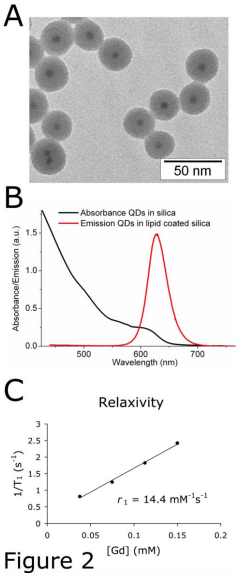

Figure 2

To assess specificity of the contrast agent, HUVEC were incubated with either RGD functionalized nanoparticles, non-functionalized nanoparticles (no RGD), or they were not incubated with nanoparticles (untreated). Fluorescence microscopy images of HUVEC incubated with RGD conjugated particles showed they were clearly associated with the cells while bare particles were marginally or not taken up by these cells (Figure 3A). Next, the cells were collected and transferred into small Eppendorf cups. Under UV illumination only the cells that had been incubated with RGD conjugated Q-SiPaLCs (Figure 3B, left cup) exhibit a bright red fluorescence, and could clearly be distinguished from the two other cell pellets (Figure 3B).

Finally, MRI was performed on these cell pellets. A T1-weighted image of the different cell pellets is depicted in Figure 3C and clearly demonstrates higher signal intensity for the pellet of cells incubated with the RGD conjugated particles as compared to the control cell pellets, which is also reflected by the decreased T1 and T2 values of the cell pellets.

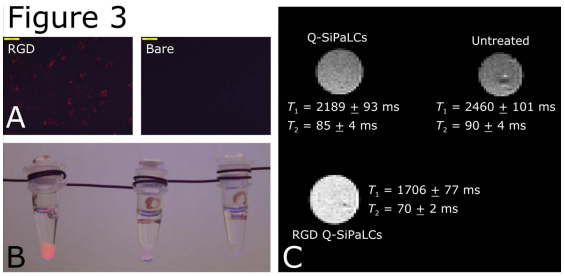

Figure 3

Conclusions

In conclusion, we have synthesized a novel type of nanoparticulate contrast agent for target-specific multimodality imaging. The nanoparticle is comprised of a QD incorporated in a silica sphere of 43 nm, which is subsequently surrounded by pegylated, paramagnetic, and biocompatible lipids. The use of the presently reported method for creating biocompatible and target-specific silica nanoparticles opens new possibilities for applying multimodality imaging contrast agents by integrating any desired combination of contrast agents and bio-fnctional groups in the silica nanoparticle.

(1) Cai W & Chen X. Small. 2007. (2) Michalet X *et al*. Science. 2005. (3) Frullano L & Meade TJ. J Biol Inorg Chem. 2007.