Multimodality imaging of gene delivery via fluoresecent iron oxide nanoparticles

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Introduction

Despite much effort and many advances in the use of viruses and synthetic systems, efficient and reliable gene transfection in vivo has yet to be established.[1] With the goal of providing a platform to improve the understanding of the gene delivery process in vivo, we developed a fluorescent iron oxide nanoparticle with a gene transfection enhancing polymeric coating (**Fig. 1**). This nanoparticle can be imaged using MRI, fluorescence and transmission electron microscopy (TEM) techniques, allowing its anatomical, cellular and sub-cellular localization to be determined. We used this nanoparticle system in the setting of liver transfection to study the effect of varying the polymer coating on the particle's pharmacokinetics, distribution and transfection ability, with the aim of controlling the nanoparticle accumulation into the therapeutically relevant hepatocytes cells.

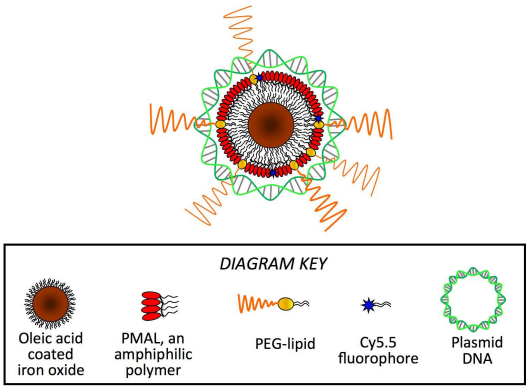

Figure 1 Schematic depiction of fluorescent iron oxide gene delivery nanoparticle

Materials and Methods

16 nm iron cores were dispersed in water using a polymaleic anhydride polymer and polyethylene glycol (PEG)-phospholipid mixed coatings, where the PEG content varied from 0-25%. These particles were termed 0% PEG, 5% PEG, 10% PEG and 25% PEG. Cy5.5 (1%) was also included in the coating. The resulting nanoparticles were characterized using TEM, dynamic light scattering (DLS), phosphorous analysis and for zeta potential. Their DNA binding properties were investigated using agarose gel electrophoresis. Their propensity to aggregate in 10% serum was investigated using DLS. Their uptake in 293T cells was visualized using confocal microscopy, TEM and MRI. Transfection with GFP was analyzed with confocal microscopy. The properties of these nanoparticles were investigated in wild type mice, via circulation half-life measurements, in vivo MR imaging, fluorescence imaging of organs and TEM on liver sections while GFP expression was probed using fluorescence analysis of liver lysates.

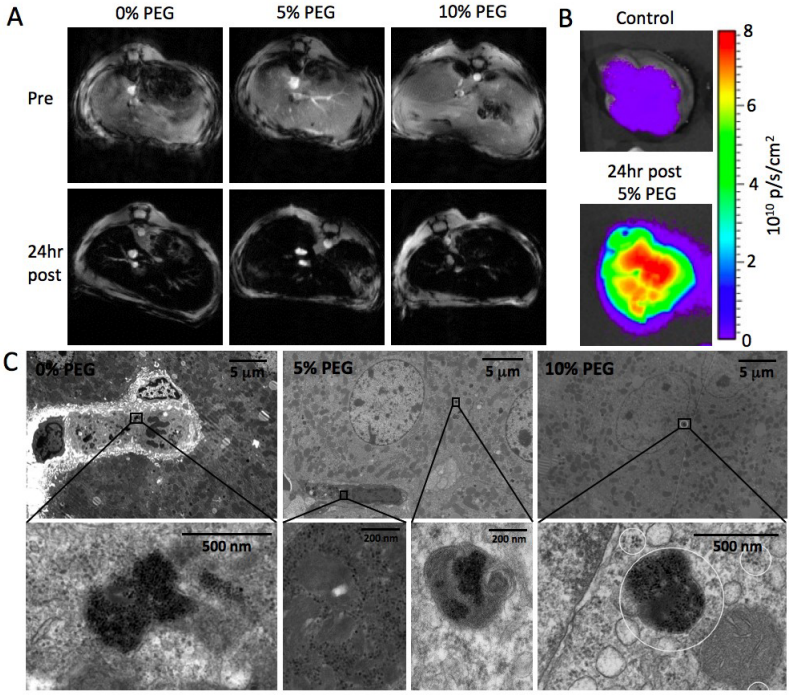

Figure 2 A) MR images of the livers of mice pre- and 24hr post-injection with plasmid carrying iron oxide nanoparticles. B) Cy5.5 channel fluorescence images of liver of mice. C) TEM images of sections of liver tissue indicating the nanoparticle cellular distribution.

Results and Discussion

As the level of PEG in the coating increased, the nanoparticle diameter increased from 19 to 32 nm. DNA was bound by the nanoparticles when PEG was 0-10% of the coating, but not when PEG was 25% of the coating. PEG prevented nanoparticle aggregation in serum. In vitro experiments with 293T cells demonstrated the potential of the nanoparticles to be imaged using MRI, fluorescence and TEM, as well as their potential to promote transfection. MRI and fluorescence imaging of the livers of mice injected with these nanoparticles showed strong contrast after 24 hours, but little difference was observed when the level of PEG in the coating was varied (**Fig. 2A** and **B**). TEM imaging, on the other hand, showed that increasing the level of PEG to lead to avoidance of the Kupffer cells and access to the therapeutically relevant hepatocytes cells. Additionally, the circulation half-life of the nanoparticles was extended from 0.2 hr with 0% PEG, to 1.8 hr with 10% PEG. Analysis of the liver lysates showed these nanoparticles to be effective for gene transfection in vivo (**Fig. 3**).

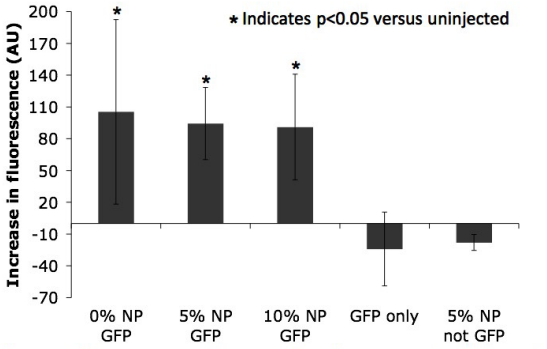

Figure 3 Fluorescence of mouse liver lysates under different conditions (AU=arbitrary units)

Conclusions

Transfection was achieved in vitro and in vivo using iron oxide NPs. The NPs have properties for MRI, fluorescence and TEM imaging. Greater levels of PEG in the NP coating resulted in extended blood half-lives and preferential accumulation in the hepatocytes, pointing to their potential as therapeutic liver transfection probes.

References

[1] Bryson, JM; Fichter, KM; Chu, WJ; Lee, JH; Li, J; Madsen, LA; McLendon, PM; Reineke, TM. Proc. Natl. Acad. Sci. USA, 2009, 106, 40, 16913-16918.