# Stearic-Polyethylenimine Modified SPIO Nanoparticles for MRI of Gene Delivery to Liver

lin Gao[1], zhiyong Wang[1], lisi Xie[2], xiaojing Long[1], zhiying Chen[3], and bensheng Qiu[1,4]

[1]*Paul C Lauterbur Research Center for Biomedical Imaging, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, China, People's Republic of,* [2]*Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Gaungzhou, Guangdong, China, People's Republic of,* [3]*Gene and Cell Engineering Center, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, China, People's Republic of,* [4]*Department of Radiology, University of Washington School of Medicine, Seattle, Washington, United States*

## *Introduction*

Gene therapy is a promise approach for treatment of various liver diseases. However, it is lacking of non-invasive imaging techniques to monitor gene delivery and gene transfer into livers. In this study, we developed stearic-PEI600- SPIO-LacZ gene for in vivo MR imaging of gene delivery to livers.

## *Methods*

For polymer synthesis, the stearic acid was reacted with 1,1-carbonyldiimidazol. Then, the carbonyldiimidazole-activated stearic acid was transferred into a flask containing a mixture of branched polyethylenimine (PEI, MW=600) and dry choloform. The suspension was stirred for 24 hours. Finally, the purified product stearic-PEI600 was obtained via precipitation in cold diethyl ether and confirmed by 1H NMR ($CDCl_3$). For superparamagnetic iron oxide (SPIO) nanoparticles synthesis, iron(III) acetylacetonate was mixed with 1,2-hexadecanediol, oleic acid, and oleylamine in benzyl ether. The mixture was heated up to reflux for 1h. The crude product was washed two times with ethanol. Then, the purified product was kept in hexane. Before surface modification, the SPIO nanocrystals were dried under argon and redispersed in a chloroform solution of amphiphilic stearic-PEI600. The solution was slowly added into water with sonication to form stearic-PEI600-SPIO nanocomplexes.

For animal experiments and MRI, BABL/c female mice (average body weight 20g) were used for MRI scanning and tissue sections at 48 hours after administrating the nanocomplexes by caudal vein injection. Mice were anesthetized with 1% pentobarbital sodium in intraperitoneal injection for MRI. Anesthetized mice were positioned in mouse coil and adopted TSE sequence. T2-weighted images (TR=3000ms, TE=48ms, FOV=25×25.5mm, slice thickness=1mm, flip angle=180) were acquired at 3.0T. All images were pre-processed with intensity normalization and histogram matching by using ITK program (www.itk.org). The mean intensity value of each ROI was calculated.

For histochemistry staining, 10 μm tissue sections were fixed for 10min with 2.5% glutaraldehyde, and then performed with Prussian blue, X-Gal and nuclear fast red staining. The pictures were taken under Leica Fluorescence Microscope. Data were presented as mean values ±stand deviation. Statistical tests were performed by using independent-samples t test (SPSS version 17.0, SPSS Inc). All statistical tests were adopted two-tailed tests and homogeneity of variance tests, considered to be significant differences if $P<0.05$.

## *Results*

The previous results showed that the stearic-PEI600-SPIO nanocomplexes can high effectively combine with LacZ DNA plasmid in vitro experiments(data not given). The T2-weighted MRI showed that the signal intensity decreased 58% at 0.5 hours and slightly recovered at 48 hours after stearic-PEI600-SPIO-LacZ injection (Fig. 1B&D). The statistical analysis showed that the T2-weighted MR signal intensity was decreased significantly in comparison with the controls ($P<0.05$) (Fig. E). The Prussian blue staining results showed that increased iron accumulation in both liver tissues and endothelial of vessels of the stearic-PEI600-SPIO-pcDNA3.1 and stearic-PEI600-SPIO-LacZ group (Fig.2C&D). The X-Gal staining results showed the illustrated β-galactosidase (LacZ gene transfected) expression in liver tissues rather than endothelial vessels in the stearic-PEI600-SPIO-LacZ group (Fig.2G&H). These initial results demonstrated that stearic-PEI600-SPIO had a lower intra-cellular clearance rate, which could penetrate the membrane barrier of endothelial system effectively for gene expression and therapy in the livers.

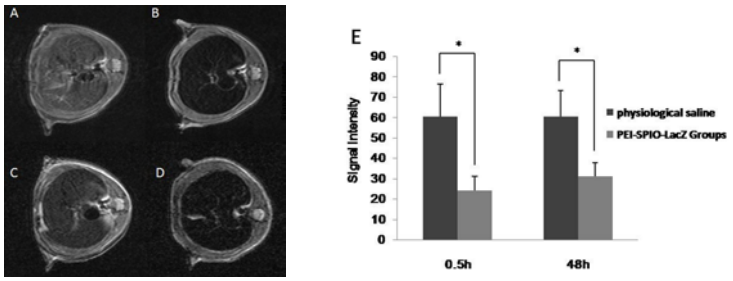


**Fig.1** The signal intensity change of T2 weighted MRI of mice livers after stearic-PEI600-SPIO-LacZ injection at 0.5h and 48h. A, C: Physiological saline injection. B, D: Stearic-PEI600 -SPIO-LacZ injection. E: Signal Intensity quantification of T2-weighted MRI (*$P<0.05$).

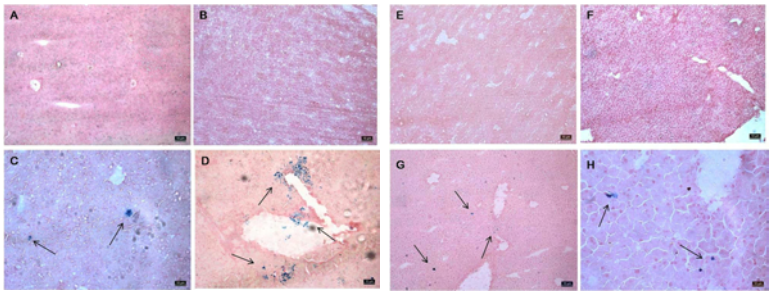


**Fig.2** X-Gal and Prussian blue staining showed β-galactosidase expression and intracellular iron accumulation in liver tissues. A: Physiological saline injection. B: LacZ gene injection alone. C: stearic-PEI600-SPIO-pcDNA3.1 injection. D: stearic-PEI600 -SPIO-LacZ injection. E: LacZ gene plasmid injection alone. F: stearic-PEI600-SPIO-pcDNA3.1 injection. G: stearic-PEI600- SPIO-LacZ injection (10×). H: stearic -PEI600-SPIO-LacZ injection (40×). The blue dots indicated either SPIO or β-gal expression in liver tissues.

## *Conclusion*

This study demonstrated that stearic-PEI600-SPIO biomaterials can combine with DNA plasmids and successfully deliver gene into target livers for non-invasive MRI of gene delivery into livers in vivo.

## *Reference*

1. Niclas E. Bengtsson, Gary Brown, Edward W. Scott et al. lacZ as a Genetic Reporter for Real-Time MRI. MRM. 2010, 63:745-753.
2. Shouheng Sun, Hao Zeng, David B. Robinson et al. Monodisperse $MFe_2O_4$ (M= Fe, Co, Mn) Nanoparticles. J. AM. CHEM. SOC. 2004, 126: 273-279.