

## **Silica-Coated Superparamagnetic Iron oxide Nanoparticles Are More Durable for Labeling Mesenchymal Stem Cells Than Poly(Ethylene Glycol)-Coated Counterparts: Pilot in-vivo Assay Results**

Y-X. Wang<sup>1</sup>, K. C. Leung<sup>2</sup>, T. Quercy-Jouvet<sup>2</sup>, H. H. Wang<sup>3</sup>, C. P. Chak<sup>2</sup>, S. Lin<sup>3</sup>, D. F. Wang<sup>3</sup>, D. W. Au<sup>4</sup>, P. C. Leung<sup>5</sup>, and K. P. Fung<sup>5</sup>

<sup>1</sup>Department of Imaging and Interventional Radiology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, <sup>2</sup>Center of Novel Functional Molecules, Department of Chemistry, The Chinese University of Hong Kong, Hong Kong, <sup>3</sup>Department of Imaging and Interventional Radiology, The Chinese University of Hong Kong, Hong Kong, <sup>4</sup>Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, <sup>5</sup>Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong

**Introduction:** MRI of superparamagnetic iron oxide (SPIO) particle labeled cells has been proposed an effective approach for non-invasive in vivo tracking of the localization and migration of the targeted cells (1-3). In some circumstances, the cells are labeled with SPIO particle in vitro, and then these cells are administered to animals or human bodies so to render them detectable with MRI. After the intracellular labeling with clinically available, large dextran-coated SPIO particles, these SPIO particles can be biodegraded by intracellular enzymes and acid (4). Silica has been proposed as a coating material for SPIO core (5). Silica has good biocompatibility and hydrophilicity and can prevent the aggregation of particles in liquids and improve their physicochemical stability. For example, Bioglass® which contains silica (~60 mol%), calcium, and phosphorus gives many applications in wound healing both in bone (hard) and soft tissues, middle ear implants, and in dentistry [6]. When silica is coated outside the SPIO core, the silica shell may be able to act as a stabilizer, limiting the effect of the outside environment, include intracellular enzymes and acids, on the core particles. In the current study, an attempt was made to compare the transfecting agent-free mesenchymal stem cell (MSCs) labeling using SPIO with different coatings, with a view to study the durability and retainability of these SPIOs in MSCs.

**Materials and methods:** Three SPIO nanoparticles, silica-coated SPIO with surface amine groups, dextran coated SPIO, poly(ethylene glycol)-coated SPIO, were tested in this study. These nanomaterials have a similar SPIO core size of 6 nm. Together with their coating, the overall sizes are 8.5, 10-15, and 10-15 nm for SPIO@SiO<sub>2</sub>-NH<sub>2</sub>, SPIO@dextran, SPIO@PEG, respectively. These SPIOs were synthesized in our own laboratories, and are hydrophilic because of the oxygen atom-rich tethers. The animal study was approved by the institutional Animal Experiment Ethics Committee. A 20-week-old male New-Zealand white rabbit was used. Bone marrow was aspirated from rabbit iliac bone and washed with Dubellco modified eagle medium (DMEM, Gibco 31600) (bone marrow:DMEM = 1:4). The mixture was spinned. Then the fat debris and supernatant was removed. The pellet was resuspended with 10% FCS (Fetal Calf Serum, Gibco 16140) in DMEM. The cell suspension was transferred to 75 cm<sup>2</sup> tissue culture flask. The cell culture was incubated at 37 °C with 5% CO<sub>2</sub>. Half of the basal medium was refreshed after 4 days and all the culture medium was refreshed after another 3 days. The adherent mesenchymal stem cells were grown in colony. The cells can be subcultured into other culture flask for cell expansion after 5-7 days. The MSCs of passage 4 were used, and cultured at same density at 6-well plate. MSCs were labeled with SPIO by incubating with FCS free DMEM culture media containing a SPIO nanomaterial overnight. Each SPIO nanomaterial was tested with various non-toxic iron concentrations. Transfection agent was not used in the study. Prussian blue staining was used to assess the labeling efficiency (i.e. iron loading) in MSCs. For this, a blueness measurement method was used [7]. For each pixel, the blueness relative to the redness and greenness was calculated. Only the pixels with relative blueness above a certain threshold were selected. Among these interested pixels, an average value of the relative blueness was calculated and taken as the blueness measure for the whole image. This blueness measure is capable of reflecting the degree of blueness in the overall image, regardless of the intensity and color in the background [7]. Based on blueness measurement values, MSCs with similar SPIO loading were selected for further culture. These cells were cultured at 37 °C with 5% CO<sub>2</sub> and normal divisions were permitted. Prussian blue staining and blueness measurement was performed again three weeks after the SPIO labeling.

**Results:** Without the application of any transfection agent, the labeling efficiency of SPIO@dextran was poor and it was dropped from the further studies. The initial blueness measurement was approximately 14 for both SPIO@SiO<sub>2</sub>-NH<sub>2</sub> and SPIO@PEG labeled MSCs cultures. At the week 3, blueness of both SPIO@SiO<sub>2</sub>-NH<sub>2</sub> and SPIO@PEG labeled MSCs decreased significantly compared with at day 0. It was also observed that SPIO@SiO<sub>2</sub>-NH<sub>2</sub> labeled MSCs had more blue staining spots than SPIO@PEG labeled MSCs (Fig 1). The blueness for MSCs labeled with SPIO@SiO<sub>2</sub>-NH<sub>2</sub> was 2.4, and that of MSCs labeled with SPIO@PEG was 0.0037.

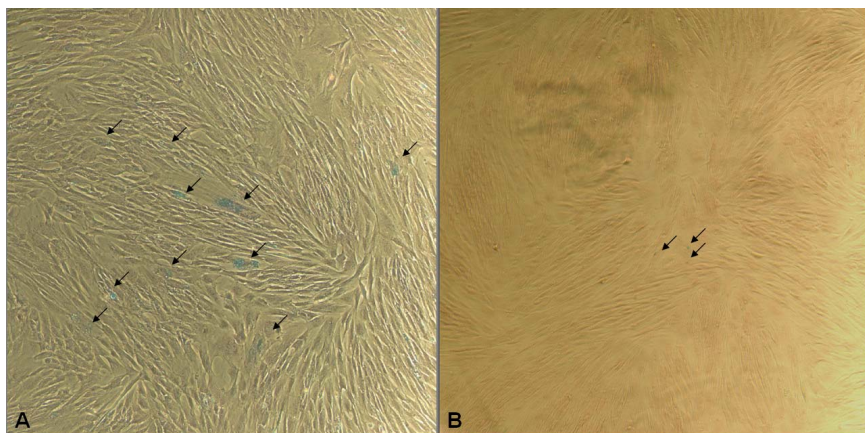


Figure 1, A: MSCs three weeks post SPIO@SiO<sub>2</sub>-NH<sub>2</sub> labeling, there are multiple blue staining iron dots (arrows). B: MSCs three weeks post SPIO@PEG labeling shows only trace amount of blue staining iron (arrows).

**Discussion and Conclusion:** An inert silica coating on the surface of magnetite nanoparticles may be able to prevent their aggregation in liquid, improves their chemical stability, and provides better protection against toxicity. The attenuation of the blueness measurement, reflecting loss of SPIO within MSCs, can be due to its biodegradation, as well as due to exocytosis and dilution of cell division. The preliminary results in this study suggest silica coating offers better protection to its SPIO core compared with biodegradable PEG coating when the nanomaterials are within MSCs, though the role of -NH<sub>2</sub> need to be further investigated. These pilot in-vivo assay results also suggest

that SPIO@SiO<sub>2</sub>-NH<sub>2</sub> will be suitable for durable stem cell labeling than biodegradable coated counterparts [8].

# the first two authors contributed equally to the work.

**References:** [1]. Wang YX, Hussain SM, Krestin GP. *Eur Radiol.* 2001;11:2319-31 [2]. Corot C, Robert P, Idée JM. et al. *Adv Drug Deliv Rev.* 2006;58:1471-504 [3]. Syková E, Jendelová P, Herynek V. *Methods Mol Biol.* 2009;549:197-215. [4]. Skotland T, Sontum PC, Oulie I. *J Pharm Biomed Anal* 2002; 28:323-329 [5]. Yan F, Xu H, Anker J, et al. *J Nanosci Nanotechnol.* 2004;4:72-6. [6]. Hench LL, Wilson J, In: Clarke ED, Simmons J, Folz D, ed. *Ceramic Transactions*, American Ceramic Society, OH, 1999, p.101. [7]. Wang YX, Leung KC, Cheung WH, et al. *J Mag Reson Imaging* 2010; 31: 1508-1513. [8]. Wang HH, Wang YX, Leung KCF, et al. *Chem. Eur. J.* 2009; 15,12417-2425.