**In Vivo Targeting of Fluorescent Silica Nanoparticles to Ischemia via Enhanced Permeability and Retention Effect**

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## Statement of Purpose

Targeted delivery of therapeutic modalities to disease has been pursued to enhance their efficacy. During past several years targeting of nanoparticles to cancer has been intensively studied for the diagnostic imaging and therapy of cancer by using characteristic leaky blood vessel around solid tumor [1,2]. Nanoparticles circulating in the bloodstream can extravasate through the leaky blood vessel around tumor and accumulate in tumor, which is called enhanced permeability and retention (EPR) effect. However there are a few reports to target nanoparticles to other disease. In this study, we report in vivo targeting of nanoparticles to mouse hindlimb ischemia using fluorescent silica nanoparticles as model nanoparticulate system.

## Methods

*Nanoparticles*: The fluorescent silica nanoparticles were prepared by silica sol-gel reaction through reverse microemulsion systems. A fluorescent dye, rhodamine B, was covalently conjugated into the silica matrix during the synthesis. The surface of the fluorescent silica nanoparticles was modified with polyethylene glycol (PEG). The pegylated nanoparticles (R-$SiO_2$-PEG) were dispersed in PBS prior to injection to mice.

*Murine hindlimb ischemic model*: Unilateral hindlimb ischemia was created on C57BL/6J mice (Jackson Laboratory, ME) according to the previous report [3,4]. In brief, the external iliac and femoral artery and vein were ligated under anesthesia and the incisions were closed by 5-0 Ethilon sutures (Johnson & Johnson, NJ). Blood flow in the hindlimb was monitored by a laser Doppler perfusion imaging (LDPI) system (Perimed AB, Sweden) and the results were normalized to the control unligated limb of the same animal.

*Injection and imaging*: The nanoparticles in 200 µl of saline were injected into the animals (n=3) intravenously at appropriate time point after surgery. Then the organs of the animals were extracted and imaged under the fluorescent dissecting microscope (Leica, IL) to check the accumulation of fluorescent nanoparticles.

## Results

The fluorescent silica nanoparticles were successfully synthesized and the pegylated nanoparticles were well-dispersed in PBS. The size of the fluorescent silica nanoparticles were 40 nm in transmission electron microscope (TEM) image (**Fig. 1a**) and 80 nm in dynamic light scattering (DLS). Rhodamine B was covalently conjugated into the silica matrix and the resulting silica nanoparticles showed fluorescent property with emission of 580 nm (**Fig. 1b**). The fluorescent imaging of the ischemic leg and normal leg of the same mouse that were injected with fluorescent silica nanoparticles (**Fig. 2**) demonstrates that the accumulation of nanoparticles in ischemic leg is much higher than in normal leg. **Fig. 3** represents the biodistribution of the fluorescent silica nanoparticles, showing higher accumulation of the nanoparticles in ischemic leg than normal leg.

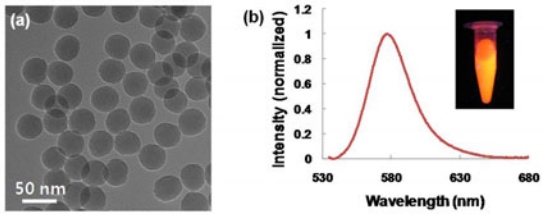


***Figure 1***. (a) TEM image and (b) photoluminescent spectra and picture of the fluorescent silica nanoparticles.

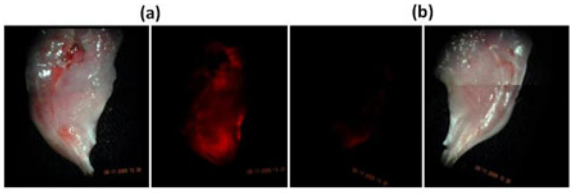


***Figure 2***. Comparison between ischemic leg and normal legs in fluorescence. (a) Bright field (left) and fluorescent (right) images of ischemic leg. (b) Fluorescent (left) and bright field (right) images of normal leg.

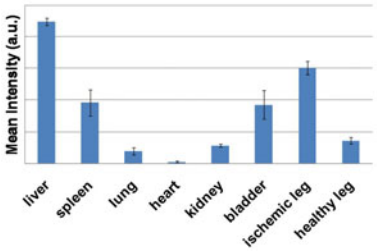


***Figure 3***. Biodistribution of the fluorescent silica nanoparticles.

## Conclusions

The EPR effect was successfully applied to *in vivo* targeting of the fluorescent silica nanoparticles to ischemia by using murine hindlimb ischemic model. The targeted accumulation of the nanoparticles in ischemic region was visualized with high fluorescent signal in fluorescent imaging.

## References

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