Supporting Information

Cerenkov radiation-mediated in situ activation of silicon nanocrystals for NIR optical imaging

Xun Zhang,[a,b#] Jingchao Li,[a#] Tingting Wang,[a,b] Nian Liu,[*a] Xinhui Su[*a]

a. PET Center, Department of Nuclear Medicine, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China.

b. School of Medicine, Xiamen University, Xiamen 361005, China.

#X. Zhang and J. Li contributed equally to this work.

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Experimental Section

1.1 Materials

^{18}F-fluorodeoxyglucose (^{18}F-FDG) and Na^{99m}TcO$_4$ were obtained from the First Affiliated Hospital, Zhejiang University School of Medicine. Silicon nanocrystals (SiNCs) was purchased from SINBIOSYS SRL (Bologna, Italia), which was synthesized by a four-step process from a previously reported method.[1] Firstly, hydrogen Silsesquioxane was went through thermal disproportionation to obtain hydride terminated SiNCs. Then dimethylvinylchlorosilane (DMVSiCl, 0.2 mL) and 4-decylbenzene diazonium tetrafluoroborate (4-DDB, 1.5 mg) were added into hydride terminated SiNCs dispersion to prepare chlorosilane terminated SiNCs, following by the Grignard nucleophilic addition to get the allyl terminated SiNCs. Finally, allyl terminated SiNCs, thiolated poly(ethylene glycol) 2000, and azobisisobutyronitrile (AIBN) were reacted in toluene at 70 °C for 4 h to get the water-soluble SiNCs.

1.2 Materials characterization

Transmission electron microscope (TEM) and high resolution transmission electron microscope (HRTEM) image of SiNCs were measured by the JEOL JEM-F200 TEM under 200 kV. Optical spectra of SiNCs were recorded with a Spectrofluorometer (Edinburgh FS5, UK). The *in vitro* and *in vivo* optical imaging were performed on IVIS spectrum (PerkinElmer) with various optical filters. The acquired images were analyzed by Living Image 4.5 software (Caliper Life Science, Hopkinton, MA).

1.3 Comparison of the radioluminescence excited by radiopharmaceutical Na^{99m}TcO4 and ^{18}F-FDG

$Na^{99m}TcO_4$ (100 μCi), ^{18}F-FDG (100 μCi), SiNCs (15 μM), the mixture of SiNCs + $Na^{99m}TcO_4$ (15 μM, 100 μCi) and the mixture of SiNCs + ^{18}F-FDG (15 μM, 100 μCi) were separately added to the 96-well plate and then imaged upon PerkinElmer IVIS Spectrum optical imaging system (Caliper Life Sciences). The parameters of the luminescence imaging were set as follows: blocked excitation, optical filters from 500 nm to 820 nm (per 20 nm) with 2 min's exposure time. To intuitively illustrate, optical phantom images from 500 nm to 820 nm (per 40 nm) were shown in Fig 2a and Fig 2c, and their corresponding radiance counts (per 20 nm) were shown in Fig 2b and Fig 2d.

1.4 Biological tissue penetration assessment

The thicknesses of 1, 2, 3 and 4 mm chicken breast were used as the tissue coverage. ^{18}F-FDG (100 μCi) and the mixture of SiNCs + ^{18}F-FDG (15 μM, 100 μCi) were separately added into a 96-well plate. Fluorescence imaging of SiNCs was performed under 500 nm's excitation. The tissue samples with different thicknesses were placed on top of the plate and imaged in a PerkinElmer IVIS Spectrum optical imaging system. The parameters of the luminescence imaging were set as follows: blocked excitation, open emission filter with 2 min's exposure time.

1.5 Cell Counting Kit-8 Assay

The cytotoxicity of SiNCs was evaluated using 4T1 murine breast cancer cells and LO-2 human liver cells. These two types of cells were sub-incubated in 96-well microplates overnight. Then various concentrations of SiNCs in DMEM medium (0 ~ 15 μM) were respectively added and co-incubated for 24 h. The Cell Counting Kit-8 assay was used to evaluate the cell viability upon treatments.

1.6 *In vivo* optical imaging

All animal experiments were operated according to the guidelines of Animal Care and Use Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, as well as approved by the Animal Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. 4T1 tumor model was constructed by subcutaneously injection of 4T1 cells (1×10^6) into the right flanks of Balb/c nude mice. The tumor volume for *in vivo* experiments was about 80 mm^3. The mouse was first intratumorally injected SiNCs and then imaged upon the fluorescence setting. After this, 200 μCi of ^{18}F-FDG were intravenously administrated on this mouse for luminescence imaging. Only ^{18}F-FDG treated mouse was used as a control group, namely Cerenkov luminescence imaging (CLI). The luminescence setting was performed with 4 min's exposure time.

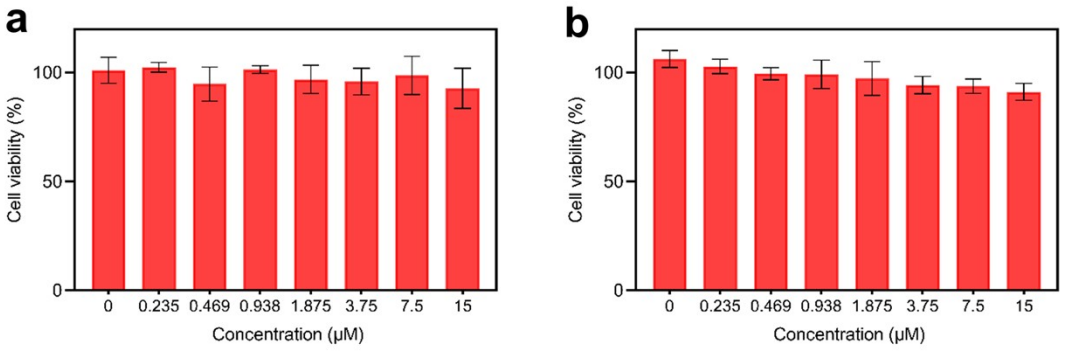

Figure S1 *In vitro* cytotoxicity of SiNCs towards (a) LO-2 cells and (b) 4T1 cells.

References

1 F. Romano, S. Angeloni, G. Morselli, R. Mazzaro, V. Morandi, J.R. Shell, X. Cao, B.W. Pogue, P. Ceroni, *Nanoscale,* 2020, **12**, 7921-7926.