Supporting Information

Iridium Complexes Nanoparticles Mediated Radiopharmaceutical-excited Phosphorescence Imaging.

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Experimental Section

1.1 Materials

2-phenylquionline (pq) and IrCl$_2$$\cdot$H$_2$O were purchased from J&K Scientific. 2,2’-bipyridyl was purchased from Energy chemical. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol was obtained from Beijing Innochem Co., Ltd. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG$_{2K}$) was purchased in Xi’an ruixi Biological Technology Co., Ltd. The other reagents were obtained from Titan. All reagents were used without further purification. Ultrapure deionized water (18.2 MΩ resistivity) from a Millipore system was used. Ir(III) complex [Ir(pq)$_2$(bpy)]Cl was synthesized according to literature.$^1$

1.2 Characterization

Transmission electron microscopy (TEM) was recorded at 200 kV with a JEOL JEM-2100 transmission electron microscope. The surface potential and hydrodynamic diameter were measured using a Malvern Zetasizer Nano ZS model ZEN3690 (Worcestershire, U.K.) equipped with a standard 633 nm laser. UV–visible absorption spectra were acquired on a Shimadzu UV-2550 ultraviolet–visible spectrophotometer using quartz cuvettes with an optical path length of 1 cm. Luminescence lifetime was performed on an Edinburgh FLS980 system excited by a 358 nm laser. Quantum yield was measured by Hamamatsu (C13534). All optical imaging measurements were performed on IVIS spectrum (PerkinElmer). All the luminescent images were acquired with various filters. The acquired images were analyzed by the Living Image 4.5 software (Caliper Life Science, Hopkinton, MA) and the signal was normalized to photons per second per centimeter square per steradian (p/sec/cm$^2$/sr).

1.3 Synthesis of [Ir(pq)$_2$(bpy)]Cl-liposomes (Ir@liposomes)$^2$

The lipid mixtures of DPPC, cholesterol, DSPE-mPEG$_{2k}$ were dissolved in chloroform and Ir(pq)$_2$(bpy)]Cl was dissolved in methanol with a molar ratio of 6:4:0.5:0.5, and then dried under a rotary evaporator. Afterwards, the dried lipid film was hydrated with 10 mL deionized water and stirred at 45 °C for 30 min. Next, after the further ultrasonication with a probe-type sonicator with a 35% amplitude for 10 min, the solution was extruded through a 0.22 μm polycarbonate filter. For further
purification, the obtained Ir@liposome was dialyzed (MWCO, 8000-14000) against deionized water for 24 h.

1.4 Comparison of the radio-phosphorescence excited by radiopharmaceutical $\text{Na}^{99m}\text{TcO}_4$ and $^{18}\text{F-FDG}$

The imaging system was the IVIS Spectrum system (Caliper Life Sciences). In order to compare the radio-phosphorescence excited by the mixture of Ir@liposome (0.4 mg/mL) plus $\text{Na}^{99m}\text{TcO}_4$ and isolated $\text{Na}^{99m}\text{TcO}_4$, $\text{Na}^{99m}\text{TcO}_4$ (40, 80, 120, 160 and 200 μCi, respectively) was added to wells in a 96-well plate, with a total volume of 250 μL. In order to compare the radio-phosphorescence excited by the $^{18}\text{F-FDG}$, mixture of Ir@liposome (0.4 mg/mL) plus $^{18}\text{F-FDG}$ and isolated $^{18}\text{F-FDG}$ were added to wells in a 96-well plate, the activity (40, 80, 120, 160 and 200 μCi respectively) with a total volume of 250 μL. The parameters of the luminescence imaging were shown as follows: blocked excitation, open emission filter with the exposure time for 5 min.

1.5 Phosphorescence and radio-luminescence imaging

The imaging system was the IVIS Spectrum system (Caliper Life Sciences). In order to compare the optical signal intensity of the mixture of Ir@liposome plus $^{18}\text{F-FDG}$, $^{18}\text{F-FDG}$, and Ir@liposome in luminescence imaging and radio-luminescence imaging, the mixture of Ir@liposome (1 mg/mL) and 50 μCi of $^{18}\text{F-FDG}$ was added in a row of 96-well plates, 50 μCi of $^{18}\text{F-FDG}$ and Ir@liposome (1 mg/mL) was added another two holes of 96-well plates. PBS was added to make sure the same volume in each hole. Ir@liposome and $^{18}\text{F-FDG}$ with the similar condition were as parallel experiments. The parameters of the IVIS Spectrum system in the luminescence imaging mode were shown as follows: binning: 4, Ex: 440 nm, Em: 580 nm, exposure: 5 s and cherenkov imaging mode with binning: 4, Ex: block, Em: open, Exposure time: 5 min.

1.6 The effect of different distance between $^{18}\text{F-FDG}$ and Ir@liposome on the intensity of radio-phosphorescence.

In the emission intensity versus excitation distance experiment, the excitation distance was set to 2, 5, 10, 15, 20 mm, respectively. The radioactivity of $^{18}\text{F-FDG}$ was 100 μCi in each acquisition, and 1mg/mL of Ir@liposome was used. The parameters
of the IVIS Spectrum system in the luminescence imaging mode were shown as follows: binning: 4, Ex: blocked, Em: open, exposure: 2 min.

1.7 Biological tissue penetration assessment

The thickness of 1, 2 and 3 mm chicken breast were used for as tissue phantom. For comparison the phosphorescence with radio-phosphorescence, $^{18}$F-FDG (100 μCi) and Ir@liposome (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively) were added to wells in a 96-well plate with a total volume of 250 μL. For comparison the Cherenkov luminescence with radio-phosphorescence, Ir@liposome (0.4 mg/mL) and $^{18}$F-FDG (40, 60, 80, 100, and 120 μCi, respectively) were added to wells in a 96-well plate, with a total volume of 250 μL. The tissue phantoms with different thickness were placed on top of the well plate and imaged in a PerkinElmer IVIS Spectrum optical imaging system. The parameters of the luminescence imaging were shown as follows: excitation wavelength: 440 nm, emission wavelength: 580 nm, exposure time: 2s. CLI and REPI were performed without blocked excitation, either open emission filter or 580 nm filter with the exposure time for 2 min.

1.8 MTT assay

HUVEC cells and 4T1 cells were obtained from Shanghai Institutes for Biological Sciences, CAS (China).

The cytotoxicity of Ir@liposome was evaluated using 4T1 murine breast cancer cells and HUVEC human umbilical vein endothelial cells. These cells ($10^5$ mL$^{-1}$) were seeded in 96-well microplates with 100 μL homologous culture medium. After 12 h of incubation, PBS and cultures including different concentrations of Ir@liposome (10, 20, 50, 100, and 200 μg/mL) were added to replace the original culture, and co-incubated for 12 or 24 h, followed by the standard MTT cell viability assay.

1.9 In vivo optical imaging

Pathogen-free seven-to eight-week-old BALB/c nude mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. All of the animals were lawfully acquired and their retention and use were in every case in compliance with federal, state and local laws and regulations, and in accordance with the Institutional Animal Care and Use Committee of SLAC (IACUC) Guide for Care and Use of Laboratory Animals. 4T1
cells (1× 10^6) were subcutaneously injected into the flanks of the animals to generate tumors. The control and experimental groups were tail-vein injected with 300 μCi of ^18^F-FDG. The tumour tissue of the REPI and PI group received direct injection of Ir@liposome solution with a dose of 0.45 mg/kg. CLI and REPI were performed with the exposure time for 5 min, and PI was performed with the exposure time for 2 s after 30 min injection.

Ir@liposome solution (0.45 mg/kg) and 30 μL of ^18^F-FDG (100 μCi) were mixed and intratumorally injected into three 4T1 mice. The control group only received the injection of Ir@liposome solution (0.45 mg/kg) intratumorally.
**Fig. S1** The hydrodynamic diameter of Ir@liposome

**Fig. S2** Time-dependent diameter changes of Ir@liposome in PBS and saline, respectively.
**Table S1** The phosphorescence lifetime and quantum efficiency of Ir@liposome and Ir(pq)$_2$(bpy)Cl under air and nitrogen atmosphere, respectively.

<table>
<thead>
<tr>
<th></th>
<th>phosphorescence lifetime (ns/Air)</th>
<th>phosphorescence lifetime (ns/N$_2$)</th>
<th>quantum efficiency (%/Air)</th>
<th>quantum efficiency (%/N$_2$)</th>
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<tbody>
<tr>
<td>Ir(pq)$_2$(bpy)Cl</td>
<td>321.3</td>
<td>400.0</td>
<td>10.3</td>
<td>14.2</td>
</tr>
<tr>
<td>Ir@liposome</td>
<td>517.5</td>
<td>596.4</td>
<td>11.8</td>
<td>16.3</td>
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**Fig. S3** The Cerenkov and radio-phosphorescence images (a) and the optical intensity of the Cerenkov and radio-phosphorescence (c) excited by $^{18}$F-FDG (40, 80, 120, 160 and 200 μCi, respectively), respectively. The Cerenkov and radio-phosphorescence images (b) and the optical intensity of the Cerenkov and radio-phosphorescence (d) excited by Na$_{99m}$TcO$_4$ (40, 80, 120, 160 and 200 μCi, respectively), respectively.
**Fig. S4** The relationship between radio-phosphorescence intensity and excitation distance.

**Fig. S5** The cell viability of HUVEC cells (a) and 4T1 cells (b) 12 and 24 h after incubation with Ir@liposome with different concentrations, respectively.
Fig. S6 The comparison between REPI and PI for in vivo imaging. (a) REPI represented radiopharmaceutical excited phosphorescence imaging of the direct intratumoural injection with the mixture of Ir@liposome (0.45 mg/kg) and $^{18}$F-FDG (100 μCi) into the 4T1 xenograft. (b) PI represented phosphorescence imaging after the direct intratumoural injection with Ir@liposome (0.45 mg/kg) into the 4T1 xenografts only. Red and yellow circle refer to the tumor. White circle refers to the muscle. (c) The optical intensity of tumor with different in vivo optical imaging. (d) The signal-to-background ratio (tumor signal intensity-background signal intensity)/background signal intensity with different in vivo optical imaging.

References