Supporting Information

In vivo Biodistribution and Clearance Studies using Multimodal ORMOSIL Nanoparticles

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TEM and DLS studies

Transmission electron microscopy (TEM) was used to determine particle size and size distribution. Aqueous dispersions of the ORMOSIL nanoparticles were dried on formvar coated copper grids (obtained from Electron Microscopy Sciences Inc.) and visualized using a JEOL JEM 2020 electron microscope. DLS (Dynamic Light Scattering) studies were carried out using a 90 plus particle size analyzer (Brookhaven).

Spectral studies

Optical absorption and steady-state photoluminescence spectroscopies were used to characterize the spectral properties of the luminescent nanomaterials. UV-Vis absorption spectra were acquired using a Shimadzu UV-3600 spectrophotometer. Photoluminescence (PL) excitation/emission spectra were recorded on a Fluorolog-3 spectrofluorimeter (Jobin Yvon, Longjumeau, France). The fluorescence life time measurements were recorded using an Easylife LS fluorescence lifetime system.

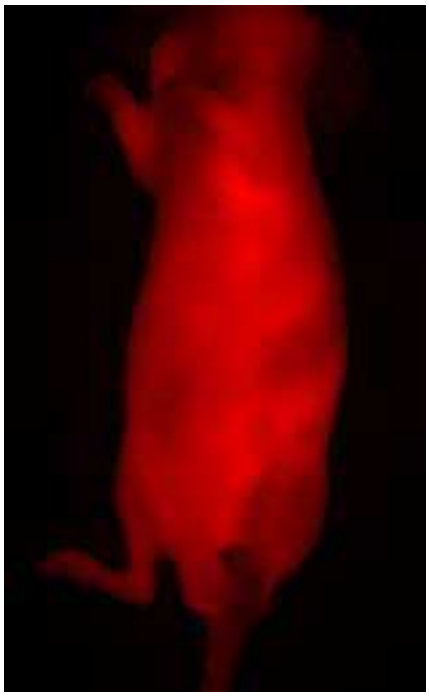
Evaluation of cell viability:

The pancreatic cancer cells, Panc 1, were dispensed into a 96-well flat-bottom microtiter plate (~10,000 cells/well) and allowed to attach overnight using DMEM medium with 10% FBS. The MTS assay has been carried out as per manufacturer's instructions (PROMEGA). It is based on the absorbance of formazan (produced by the cleavage of MTS by dehydrogenases in living cells), the amount of which is directly proportional to the number of live cells. In brief, after 24h treatment with DY776 conjugated ORMOSIL nanoparticles at varying concentration, the media was changed and 150 μL of MTS reagent was added to each well and well mixed. The absorbance of the mixtures at 490 nm was measured. The cell viability was calculated as the ratio of the absorbance of the sample well to that of the control well and expressed as a percentage. Tests were performed in quadruplicate. Each point represents the mean ±SD (bars) of replicates from one representative experiment.

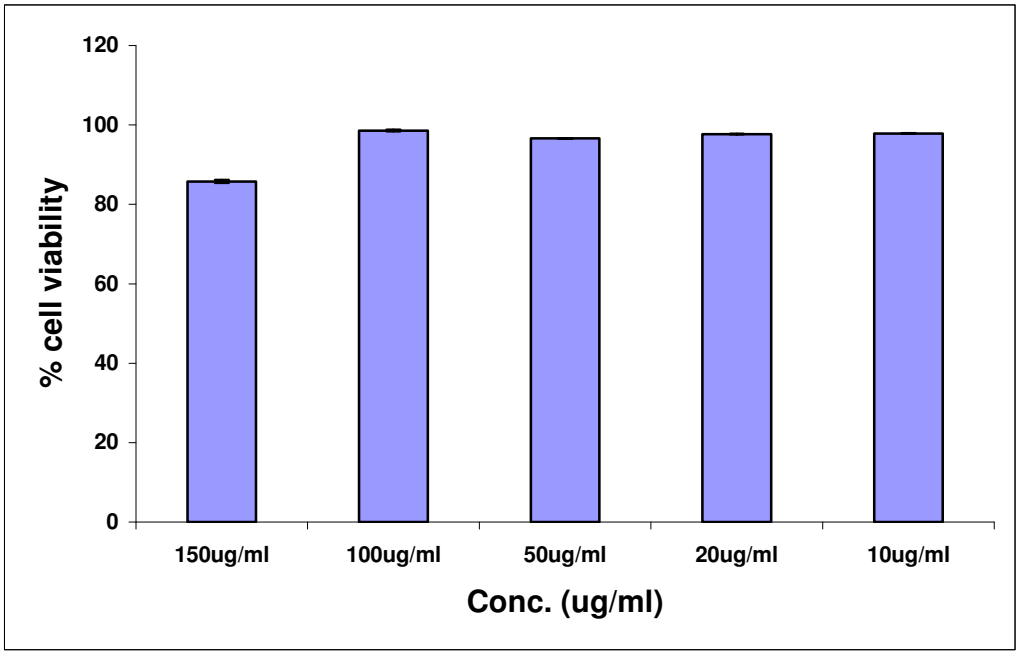
***In vivo* imaging of DY 776 conjugated ORMOSIL nanoparticles:**

In vivo fluorescence imaging was accomplished by using a Maestro GNIR FLEX fluorescence imaging system from (CRi). The nanoparticles were excited at 760nm by the defocused emission from the fiber coupled laser diode. Emission filter (800 SP) was used to cut off excitation light.

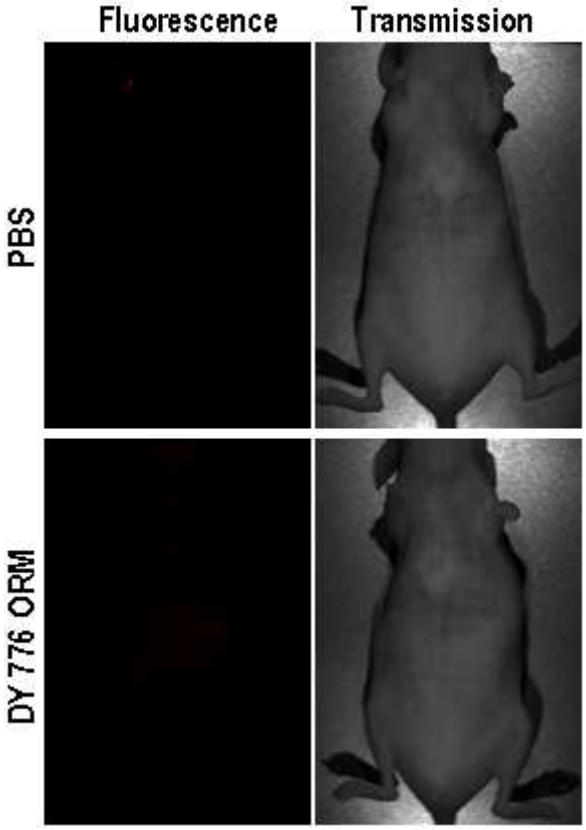
Wavelength-resolved in vivo spectral imaging was carried out by using a spectral imaging system comprising of an optical head, an optical coupler and a cooled, scientific-grade monochrome CCD camera, along with image acquisition and analysis software. The tunable filter was automatically stepped in 10-nm increments from 800 to 950 nm while the camera captured images at each wavelength with constant exposure. Overall acquisition time was about 10s. The 20 resulting TIFF images were loaded into a single data structure in memory, forming a spectral stack with a spectrum at every pixel. With spectral imaging software, small but meaningful spectral differences could be rapidly detected and analyzed. Autofluorescence spectra and DY 776 spectra were manually selected from the spectral image using the computer mouse to select appropriate regions. Spectral unmixing algorithms (available from CRi) were applied to create the unmixed images of 'pure' autofluorescence and 'pure' phosphorescence signal. When appropriately generated, the autofluorescence image should be uniform in intensity regardless of the presence or absence of phosphorescence signal. The identification of valid spectra for unmixing purposes need only be done initially, as the spectra can be saved in spectral libraries and reused on additional spectral stacks.



Supporting figure S1: Fluorescence image of the mice injected i.v. with free DY776 dye showing the dominance of the fluorescence from skin. Mice was imaged 24hrs post injection.



Supporting figure S2: Cell viability assay of DY776 conjugated ORMOSIL nanoparticles with pancreatic cancer cell line Panc 1. The incubation time was 24hrs.



Supporting Figure S3: Fluorescence and transmission images of the mice injected intravenously with PBS (upper panel) and DY776 ORMOSIL nanoparticles. Imaging was done 42 days post i.v. injection and unmixed for acquiring the DY776 fluorescence signal.