Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Title *In vivo* Biodistribution of Stable Spherical and Filamentous Micelles Probed by High-sensitivity SPECT

Laurence Jennings, Oleksandra Ivashchenko*, Irene J. C. Marsman, Adrianus C. Laan, Antonia G. Denkova, Gilles Waton, Frederik J. Beekman, François Schosseler, Eduardo Mendes*

TABLE OF CONTENTS

1. *IN VITRO* CHARACTERIZATION OF PS-PEO MICELLES

1.1 Methods

in vitro model

The evaluation of the carriers' *in vitro* was carried out using HeLa cancer cells. These were cultured in full DMEM medium containing 10% FBS and 100 units/mL penicillin-streptomycin. The cells were kept at 37°C in humidified atmosphere (5% CO_2 in air). The cells were harvested by trypsinization with trypsin-EDTA.

Cytotoxicity

The cytotoxicity of the PS-b-PEO micelles was determined using the WST-1 ver. 16 (Roche Life Science, Switzerland) colorimetric assay. WST-1 contains the 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium salts which are cleaved to formazan by cellular enzymes. Increase in the number of viable cells results in an increase of enzymatic activity, which translates to an increased amount of formazan dye. The amount of formazan dye was measured against blank samples in 96 well plates using a Powerwave XS multiwell spectrophotometer (Biotek, United States). The absorbance of the formazan product was measured at 440 nm. For each micelle sample four different concentrations, from 0.005 mg/mL to 5 mg/mL, were incubated for 24, 48 and 72 hours.

Fluorescence uptake

Cells were seeded in 6-well plates, each containing one borosilicate glass slide 20 x 20 mm, at a density of 5 x 10^5 cells/well in 1.5 mL of medium. These were incubated at 37°C and 5% CO_2 for 24 hours. After this, 300 uL of micelle solution was added to each well to obtain a final concentration of 1.3 mg of micelles per well. The samples were left for different incubation times, after which the coverslips were rinsed with PBS to remove all non-uptaken micelles and sealed on a glass slide using transparent Roti® liquid barrier marker. Five uL of Vectashield-DAPI were added between the coverslip and the glass slide to stain the nuclei and preserve fluorescence in the sample. Confocal images of the samples were made using a LSM 710 confocal microscope with a Fluar 40x/1.30 Oil M27 objective (Carl Zeiss Microscopy GmbH, Germany)**.** The DAPI signal was excited using a 405 nm diode (emission 460 nm), while the DiI using a 543 nm He-Ne laser (emission 563 nm). From confocal micrographs of the cells, the

average DiI signal per cell was measured. Control samples with no added micelles were used for background correction.

2. SUPPLEMENTARY FIGURES AND TABLES

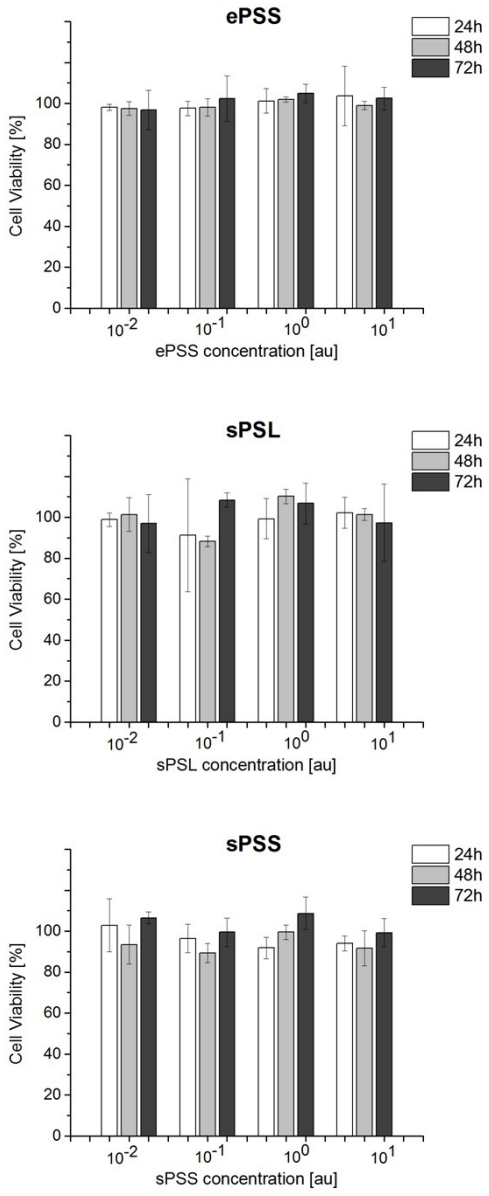

Figure S1. *Cell viability for the three micelle morphologies. Four concentrations, from 0.005 mg/mL to 5 mg/mL were tested for three incubation times: 24, 48 and 72 hours. No cytotoxic effects are visible when using the micelles in these doses.*

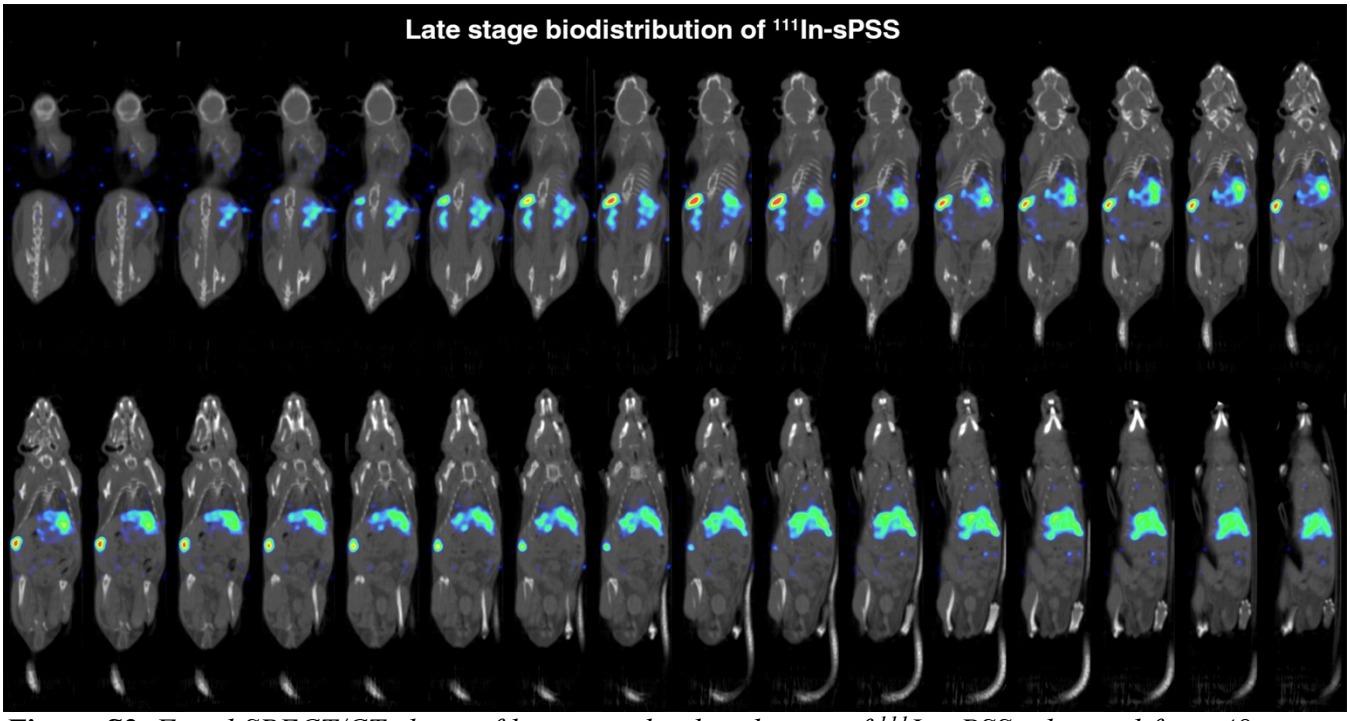

Figure S2. *Fused SPECT/CT slices of late stage biodistribution of ^{111}In-sPSS, obtained from 48 h p.i. SPECT scan with focusing on abdominal area of the animal.*

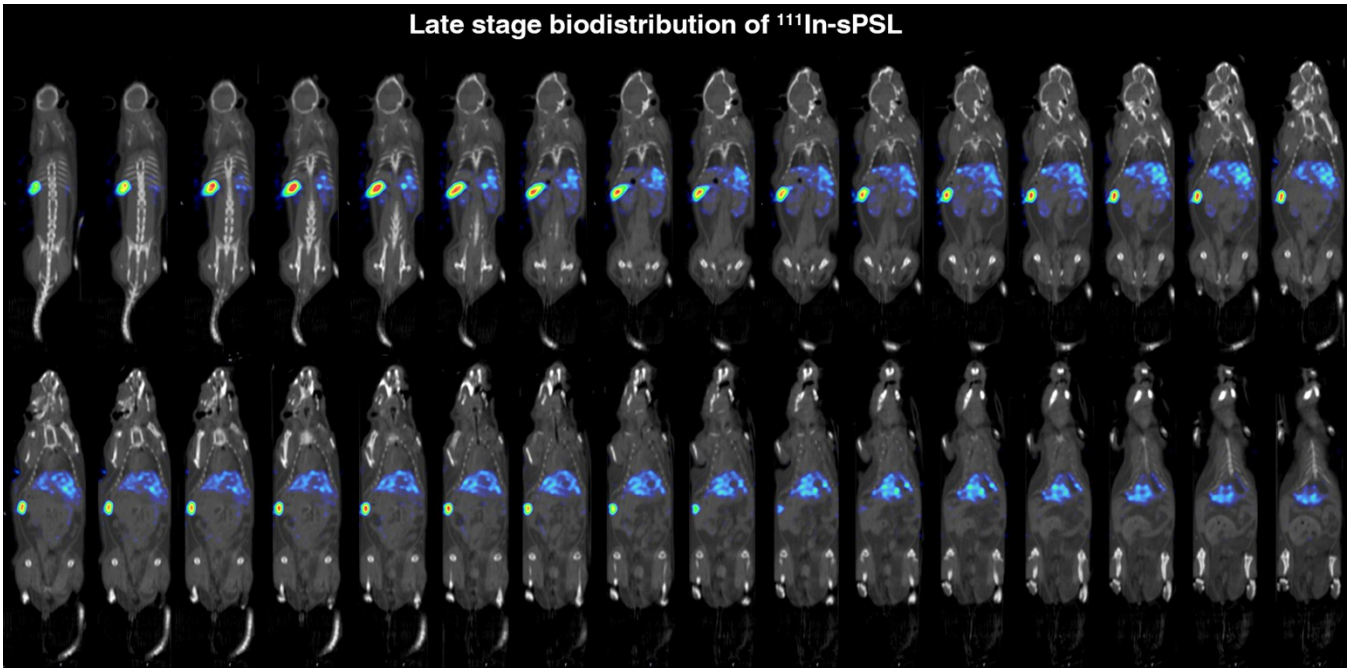

Figure S3. *Fused SPECT/CT slices of late stage biodistribution of ^{111}In-sPSL, obtained from 48 h p.i. SPECT scan with focusing on abdominal area of the animal.*

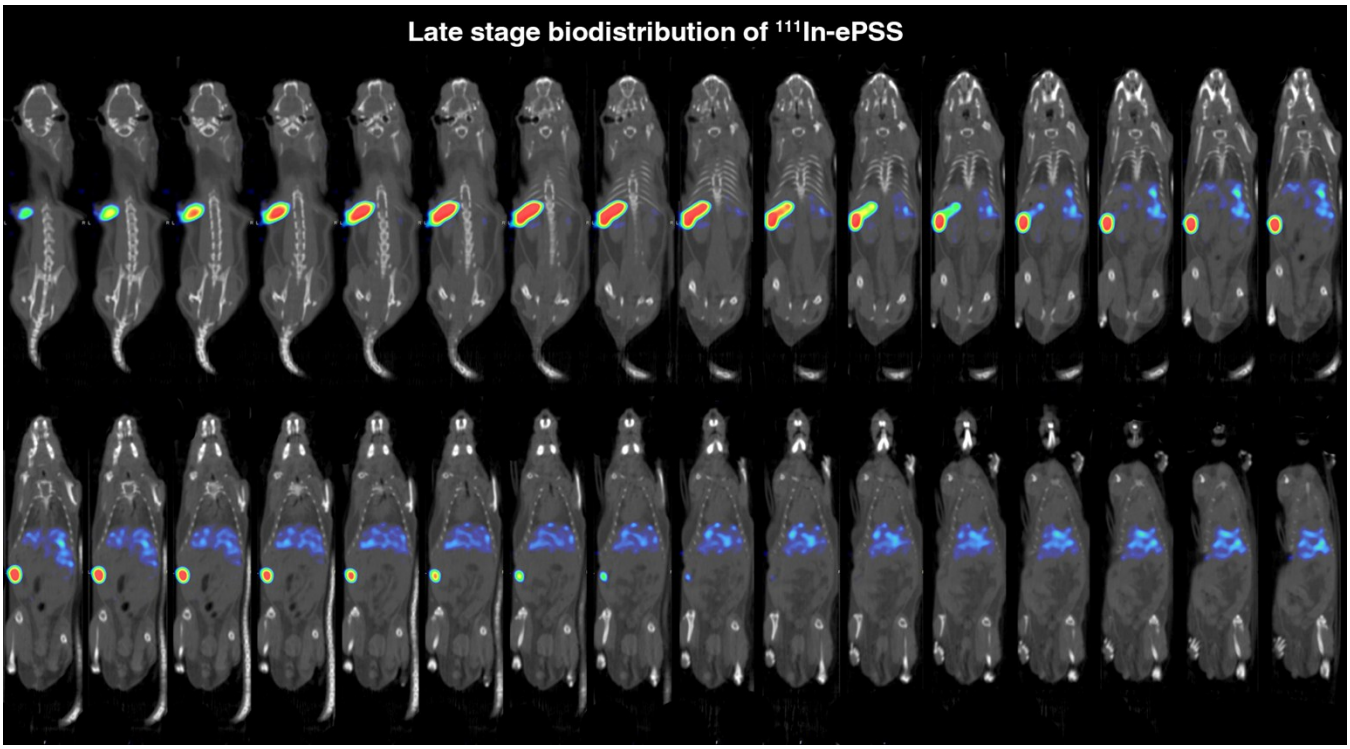

Figure S4. *Fused SPECT/CT slices of late stage biodistribution of ^{111}In-ePSS, obtained from 48 h p.i. SPECT scan with focusing on abdominal area of the animal.*

Table S1. Radiolabeling efficiency of the different micelles.

Micelle type	**Encapsulation efficiency**
^{111}In-sPSS	13%
^{111}In-sPSL	30%
^{111}In-ePSS	22%

Table S2. Comparison of accumulation rates of ^{111}In-labelled sPSS, sPSL and ePSS micelles in the heart, liver, spleen, brain and lungs of the animals at 0, 24 and 48 h p.i..

Organ	**Compound**	**Uptake [%ID/mL]**		
		0 h	**24 h**	**48 h**
Heart	^{111}In-sPSS	17.7±3.7[a)]	6.1±2.2	3.0±0.4
	^{111}In-sPSL	17.4±6.1	4.2±0.6	2.4±0.1
	^{111}In-ePSS	15.0±3.6	4.6±1.3	2.7±0.2
Liver	^{111}In-sPSS	10.4±1.9	11.8±0.4	12.5±0.8
	^{111}In-sPSL	7.9±1.7	9.6±0.2	12.3±1.3
	^{111}In-ePSS	8.8±1.5	13.5±8.0	10.1±3.0
Spleen	^{111}In-sPSS	12.2±4.4	14.3±1.8	17.3±0.9
	^{111}In-sPSL	11.1±1.4	37.5±5.1	48.3±18.2
	^{111}In-ePSS	24.7±0.4	62.0±21.9	88.2±7.5
Brain	^{111}In-sPSS	1.8±0.4	1.1±0.2	0.9±0.1
	^{111}In-sPSL	1.7±0.1	0.8±0.1	0.5±0.1
	^{111}In-ePSS	1.9±0.3	1.0±0.4	0.8±0.1
Lungs	^{111}In-sPSS	13.6±2.1	4.5±1.2	2.1±0.1
	^{111}In-sPSL	13.2±1.2	3.1±0.3	1.6±0.1
	^{111}In-ePSS	10.6±3.0	3.4±1.0	2.0±0.2

[a)]Standard deviation of the uptake within one study group of the animals

Table S3. Total uptake of ^{111}In-labelled sPSS, sPSL and ePSS micelles in the heart, liver, spleen and kidneys of the animals.

Organ	Compound	Uptake [%ID]		
		0 h	24 h	48 h
Heart	^{111}In-sPSS	5.6±0.1[a)]	2.0±0.4	1.0±0.1
	^{111}In-sPSL	6.1±1.5	1.8±0.3	0.9±0.1
	^{111}In-ePSS	5.9±0.3	1.7±0.3	0.9±0.1
Liver	^{111}In-sPSS	16.4±1.3	21.5±2.9	21.1±3.7
	^{111}In-sPSL	12.8±1.1	22.5±6.2	22.5±4.6
	^{111}In-ePSS	13.2±1.9	13.9±0.1	15.6±0.1
Spleen	^{111}In-sPSS	1.5±0.4	1.9±0.7	3.5±0.4
	^{111}In-sPSL	2.4±1.1	9.6±2.1	10.6±2.7
	^{111}In-ePSS	7.8±0.3	24.4±1.3	25.4±0.7
Kidneys	^{111}In-sPSS	3.5±0.6	3.4±0.4	2.8±0.3
	^{111}In-sPSL	3.7±0.4	4.3±0.5	3.3±0.4
	^{111}In-ePSS	4.2±1.4	2.9±0.5	2.6±1.0

[a)]Standard deviation of the uptake within one study group of the animals

3. SUPPLEMENTARY MULTIMEDIA FILES

Video S1. Animated maximum intensity projections of follow-up SPECT/CT scans with ^{111}In-sPSS, illustrating longitudinal changes in compound's biodistribution

Video S2. Animated maximum intensity projections of follow-up SPECT/CT scans with ^{111}In-sPSL, illustrating longitudinal changes in compound's biodistribution

Video S3. Animated maximum intensity projections of follow-up SPECT/CT scans with ^{111}In-ePSS, illustrating longitudinal changes in compound's biodistribution