Supplementary Information

Cellular interactions of surface modified nanoporous silicon particles

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Supplementary Information 1

Synthesis of ^{125}I-radiolabeled HFBII

The *N*-succinimidyl-3-(hydroxyphenyl)propionate (SHPP) was synthesized from 3-(4-hydrophenyl)propionic acid and *N*-hydrosuccinimide as described previously.[1]A volume of 50 µL of 1 mg/mL HFBII in 100 mM sodium borate buffer (pH 8.5) was added to dry ^{125}I-SHPP and the reaction mixture was incubated at 40 °C for 45 min. The HFBII-^{125}I was then purified on a PD MiniTrap G-25 column (GE Healthcare, USA) preconditioned with 8 mL McIlvaine buffer (pH 4.0). The product was eluted with 3 mL of the buffer collecting 200 µL fractions. Fractions were measured on a dose calibrator (VDC-405, Veenstra Instruments, the Netherlands) and peak fractions pooled. Immediately after pooling, 0.1 mg of HFBII carrier in McIlvane buffer was added to the HFBII-^{125}I solution to prevent adsorption to the vial walls. Coating of the THCPSi nanoparticles was carried out analogously to the conditions described for the standard protocol, except that the coating solution was spiked with 0.5 MBq of the HFBII-^{125}I tracer in McIlvaine buffer (pH 4.0). A ratio of 1:4 (w/w) of particles to HFBII was maintained. The stability of the HFBII-^{125}I coating on THCPSi nanoparticles was investigated with incubations in 1×HBSS (pH 7.4) at +37 °C. For the stability experiment, 0.8-0.1 MBq of fresh THCPSi-HFBII-^{125}I particles were suspended in 5 mL of the buffer and 200-µl samples were drawn at the designated time points from 0 to 360 minutes. The nanoparticles were pelleted with centrifugation at 15000*g* for 10 minutes and the radioactivities in the supernatant and particle pellet measured. The supernatants were further analyzed with paper chromatography followed by digital autoradiography as described above to corroborate that the released radioactive species was intact HFBII-^{125}I.

Supplementary Information 2

Drug loading into THCPSi nanoparticles

The drug loading into the particles was conducted prior to the HFBII coating. Initially, a batch of THCPSi-HFBII-^{125}I nanoparticles with a radioactivity concentration of 19.3 kBq/mL was used. For the loading with IMC, the particles were immersed into a drug loading solution of 15 mg/mL IMC dissolved in ethanol, with a ratio of approximately 3 mL of drug solution per 400 μg of particles. The loading time was 2 h, after which the particles were centrifuged and the supernatant (loading solution) removed. The particles were then washed 3 times with MiliQ-water and re-dispersed in HBSS (pH 7.4) and part of the batch was coated with the HFBII according to the coating protocol and used together with the uncoated particles in the permeability experiments. The average loading degree of IMC was determined by immersing the loaded nanoparticles in 5 mL of an ethanol solution for 2 h, and then quantifying the drug concentration in the solution by HPLC.

Supplementary Information 3

Bicinchoninic acid (BCA) protein quantification

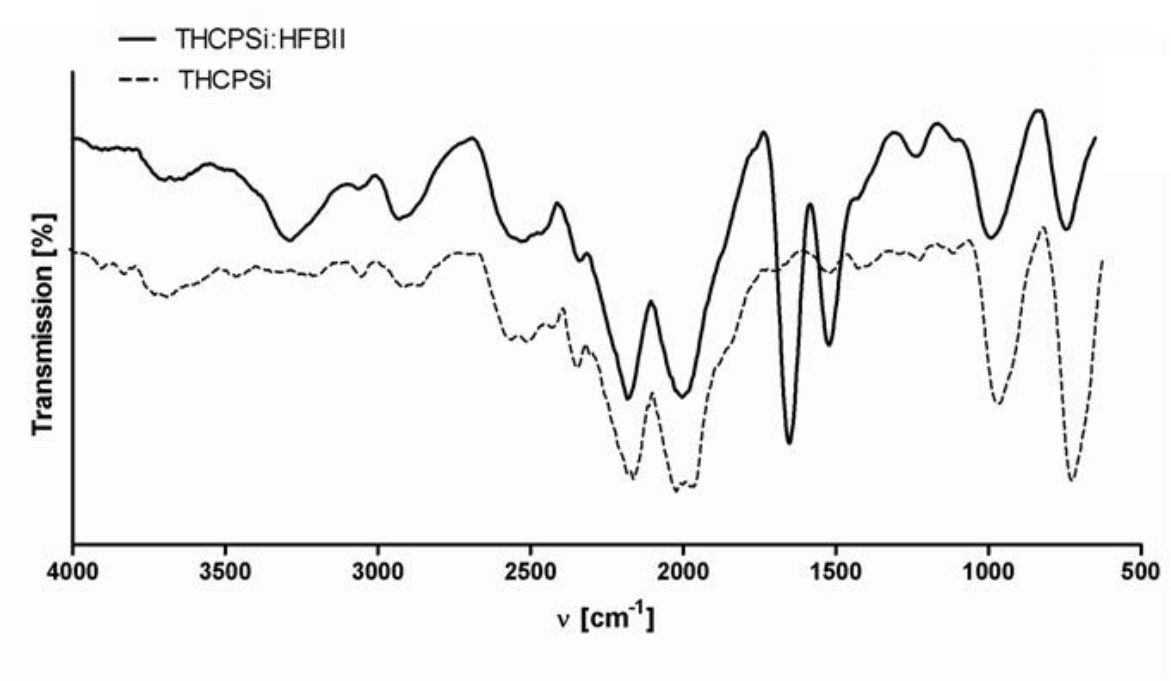
Brifely, 100 µL of each sample and appropriate standards were pippeted to a glass tube and 2.0 mL of working reagent was added and subsequently mixed. The tubes were then incubated at 60 °C for 30 min and afterwards cooled at room temperature. The absorbance of the samples was read in a Ultrospec II spectrophotometer (LKB Biochrom, UK) at a wavelenght of 562 nm and the total protein amount was calculated from a standard curve prepared from the protein standards.

Supplementary Information 4

Cell culture

The HT-29 and Caco-2 cells were cultured in 75 cm^2 culture flasks (Corning Inc. Life Sciences, USA) using Dulbecco's modified Eagle's medium (DMEM, EuroClone S.p.A., Italy) supplemented with 10% fetal bovine serum (FBS, Gibco, Invitrogen, USA),1% nonessential amino acids,1% L-glutamine, penicillin (100 IU/mL), and streptomycin (100 mg/mL) (all from EuroClone S.p.A., Italy). The cultures were maintained in a BB 16 gas incubator at 37 °C (Heraeus Instruments GmbH, Germany) in an atmosphere of 5% CO_2 and 95% relative humidity. The growth medium was changed every other day until the time of use. HT-29 from passages 30–34 and Caco-2 cells from passage numbers 31–40, were used in the experiments. Prior to each test, the cells were harvested using 0.25% (v/v) trypsin–ethylenediamine tetraacetic acid (EDTA)–phosphate buffer solution (PBS) and seeded in the desired density. For the toxicity assessment, 100 μL of a 2×10^5 cells/mL solution in DMEM were seeded in 96-well plates (PerkinElmer Inc., USA) and allowed to attach overnight. The medium was aspirated and 100 μL of uncoated and HFBII-coated THCPSi nanoparticle suspensions with concentrations of 500, 250, 50 and 15 μg/mL were added to the wells. After 6 and 24 h of incubation, the wells were washed twice with HBSS and 50 μL of fresh HBSS was added to the wells followed by the addition of 50 μL of the CellTiter-Glo™ reagent assay (Promega Corporation, USA). In this assay, the number of viable cells in culture is quantified based on the amount of ATP produced by metabolically active cells. Thus, the amount of ATP produced is directly proportional to the number of living cells presented in the culture. The plate was measured for luminescence using a Varioskan Flash fluorometer (Thermo Fisher Scientific, USA). All assays were carried out at least in triplicate.

Supplementary Information 5



Supplementary Information 5 ATR-FTIR transmission spectra of uncoated (dashed black line) and HFBII-coated THCPSi nanoparticles (solid black line). The THCPSi spectrum was shifted down for clarity.

References

1. A. E. Bolton and W. M. Hunter *Biochemical Journal*, 1973, **133**, 529-538.