SUPPLEMENTARY INFORMATION

Microfluidic Assembly of Multistage Porous Silicon/Lipid Vesicles for Controlled Drug Release

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ESI 1: Morphological changes over time of the THCPsiMPs-Lipid vesicles during release experiments

No morphological changes are observed in the delivery vehicles over time at 37 °C and in buffered conditions (pH = 7.4) during more than 200 min. Significant breakage of the delivery vehicles was observed after that time period.
ESI 2: THCPSiMPs-Lipid vesicles cell viability experiments.

To test the biocompatibility of the THCPSiMPs-Lipid vesicle system we have performed a cell viability test using an ATP-based luminescent assay. HT-29 cancer cells, obtained from a colorectal adenocarcinoma (American Type Culture Collection, USA), were selected as a model cell line. We have tested and compared the viability of these cells when seeded with different concentrations of THCPSiMPs and THCPSiMPs-Lipid vesicle, for 3 h.

HT-29 cells were cultured until passage #35, using Dulbecco’s modified Eagle’s medium (DMEM, EuroClone S.p.A., Italy) with 4.5 g/L glucose and supplemented with 10% 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.). The cell cultures were maintained in a standard incubator (BB 16 gas, Heraeus Instruments GmbH, Germany) at 37 °C with an atmosphere of 5% CO2 and 95% relative humidity.

The THCPSiMPs-Lipid vesicles were produced as described before and collected in a vial containing 100 mOsm/L sucrose, in order to allow the precipitation of the double emulsion droplets containing the THPSiMPs. The droplets were collected from the bottom of the vial using a Pasteur pipette, and were then put inside a pre-washed dialysis membrane to allow the complete evaporation of the organic solvents that were released from the structure of the lipid vesicles and remained in the outer medium. The membrane was closed tightly and immersed in a 100 mOsm/L solution inside an orbital incubator set at 37 °C for 3 h. The content of the dialysis bag, which carried a known concentration of THCPSiMPs-Lipid vesicles, was used to prepare the dilutions of different particle concentrations of particles (0.5, 0.2, 0.1, and 0.05 mg/mL) in HBSS pH 7.4. The same dilutions were prepared for the bare THCPSiMPs.

For the cell viability analysis, 100 μL of 5.0 × 10⁵ cells/mL of HT-29 in DMEM suspension were seeded in 96-well plates (PerkinElmer Inc., USA) and allowed to attach for 24 h. The medium was aspirated and the wells were washed twice with fresh 1 × Hanks balanced salt solution (HBSS, pH 7.4). Subsequently, 100 μL of THCPSiMPs and THCPSi-Lipid vesicles suspensions with concentrations of 0.5, 0.2, 0.1, and 0.05 mg/mL were added into the wells. 1 × HBSS (pH 7.4) and Triton X-100 were used as positive and negative controls, respectively. The cells with the particles were incubated for 3 h. After incubation, the wells were washed once with 1 × HBSS (pH 7.4) and the number of viable cells was determined with CellTiter-Glo (Promega Corporation, USA) following the manufacturer’s instructions. The luminescence was measured on a Varioskan Flash fluorometer (Thermo Fisher Scientific, USA). All the assays were carried out at least in quadruplicates.