Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Development of arginine based nanocarriers for targeting and treatment of intracellular *Salmonella*

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Methods

Materials

High purity Tetraethyl orthosilicate (TEOS), Protamine sulphate, Pectin, Ciprofloxacin hydrochloride (Cip) and Dulbecco's modified Eagles' medium (DMEM), fetal bovine serum (FBS), Chlorpromazine, Amiloride, Genistein, Cytochalasin D, iNOS inhibitor, N-([3-(Aminomethyl)phenyl]methyl)ethanimidamide dihydrochloride (1400w) were purchased from Sigma-Aldrich, India and used without further purification. Cetyltrimethylammonium bromide (CTAB), NaOH, NaCl and Orthophosphoric acid were purchased from SRL, India. Spectroscopy grade absolute ethanol was purchased from Commercial Alcohols, Canada. The water used in all the experiments was obtained from the Milli-Q system with resistivity greater than 18MΩcm.

Loading and release of ciprofloxacin from the nanoparticles

Arg–MSN (2 mg) was incubated with 2.5 mg/ml Ciprofloxacin (Cip) solution for 12h at 25°C. The dispersion was centrifuged to separate the particles, and the concentration of Cip in the supernatant was measured using ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, USA). The release studies was carried out in dynamic conditions using a Rotospin operating at 20 rpm at 298 ± 5 K. 6 mg Ciprofloxacin loaded Arg-MSN (Cip Arg-MSN) was transferred initially into a releasing medium 0.15 M NaCl of pH 3 for 1h and subsequently into PBS buffer solution of pH 7.4 to simulate the gastrointestinal tract environment. At regular intervals, the concentration of Cip was measured. Every 8h, the samples were centrifuged and transferred to a fresh release medium to avoid saturation of release medium. Ciprofloxacin release in the dissolution medium was measured for 24h and cumulative release was determined.

Cellular toxicity studies of Arg-MSN

Cellular viability of Arg–MSN was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. RAW 264.7 cells and HeLa cells were seeded at a density of 20,000 cells/well in a 96-well microtitre plate. The cells were treated with Arg-MSN particles at a concentration of 20 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml. After 24h, 20 µL of MTT dye (5 mg/mL) was added to each well and incubated for 4h at 37°C. Viable cells reduce the tetrazolium compound to insoluble formazan, which dissolves in DMSO to give a purple solution. The absorbance of the DMSO treated samples were measured at 570 nm. The relative absorbance of the colored solution is used as a relative measure of percentage viability.

Hemolysis assay of Arg-MSN

The hemolytic assays of amorphous nano silica, mesoporous silica and Arg–MSN particles were carried out. Blood was drawn by trained medical professional with the informed consent from healthy volunteers. 10 ml of blood was withdrawn and mixed with 1 ml of 10% EDTA to prevent coagulation. 1 ml of blood was diluted with 1 ml of 0.9 % NaCl and centrifuged at 3000 rpm for 5 min. The supernatant was removed and the sample was washed twice with 0.9% NaCl to remove plasma. The resultant pellet was dispersed in 10 ml of 0.9% NaCl solution from which aliquots of 300 µL was taken, its volume made upto 1.5 ml with the test sample and incubated for 8h. The test samples were dispersed in 0.9 % NaCl. Plain water and 0.1% SDS were used as the positive controls and 0.9% NaCl solution was used as the negative control. The samples were centrifuged at 10,000 rpm for 10 min to obtain the supernatant and percentage hemolysis determined by measuring the absorbance of the supernatant at 415 nm using Nanodrop spectrophotometer.

% Hemolysis = (Test sample – Negative control)/ (Positive control – Negative control)

Results

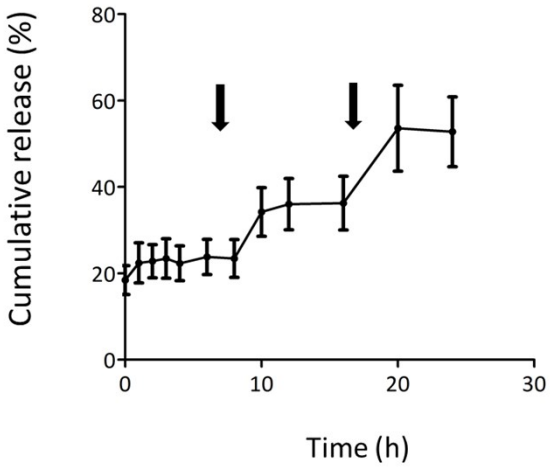

Figure S1. Release studies of ciprofloxacin from Arg-MSN (6 mg) particles for 24 h. Release was carried out at pH 3 for the first hour and transferred to PBS subsequently. Supernatant was replaced every 8 h (arrows).

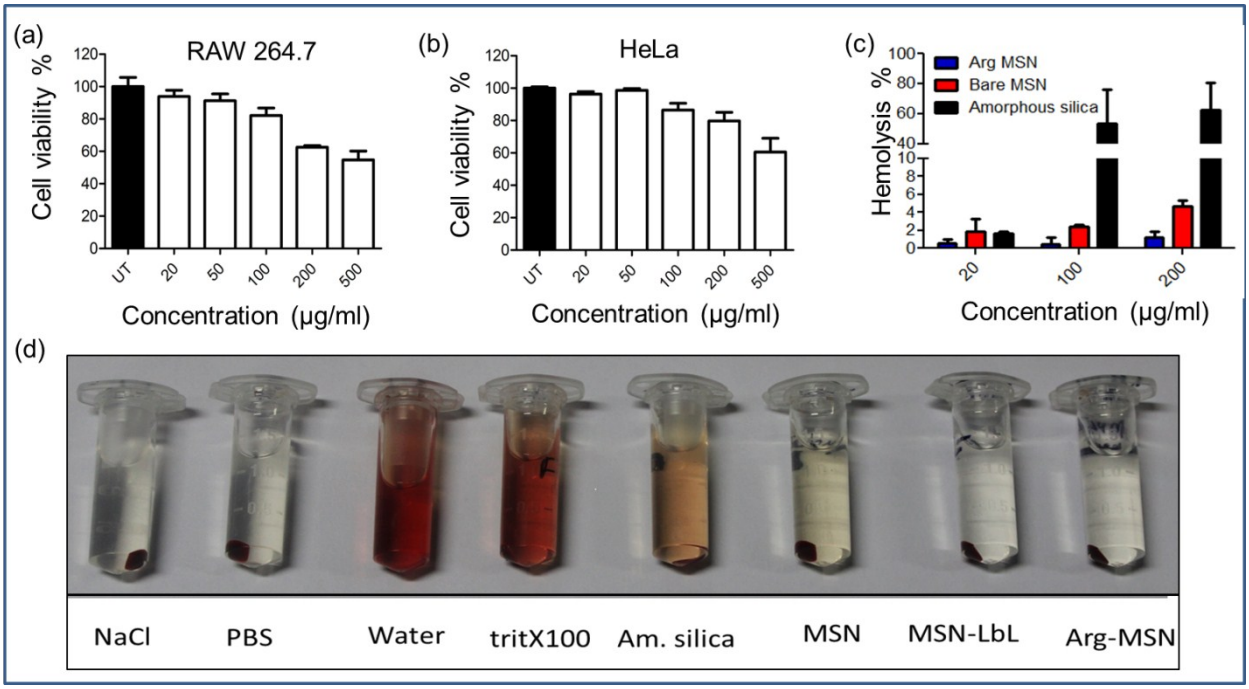

Figure S2. Analysis of biocompatibility of the Arg-MSN by MTT and hemolysis assay. (A) and (B) MTT assay of Arg-MSN in RAW 264.7 and HeLa cells respectively (C) Hemolytic assay after treated bare MSN, Arg - MSN and amorphous silica nanoparticles (fumed silica); (D) representative image of hemolysis study with 200 µg/ml of nanoparticles.

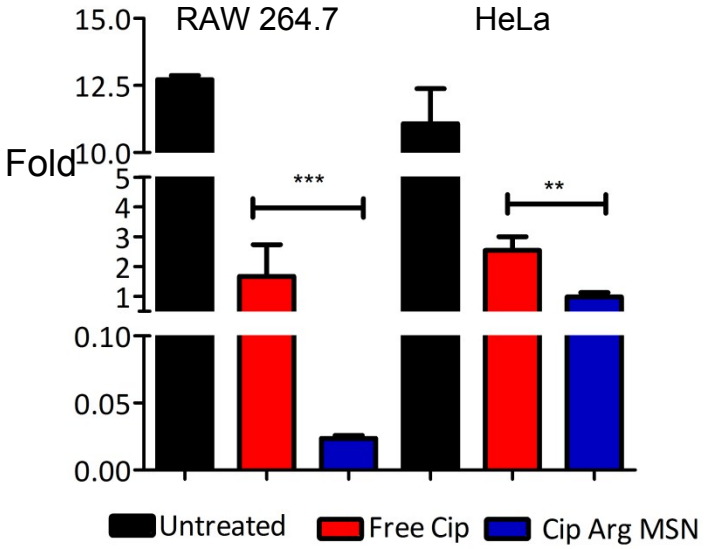

Figure S3. Evaluation of intracellular proliferation of STM with Cip Arg-MSN treatment Fold change of STM proliferation in HeLa and RAW 264.7 cells after treatment with 10 μg/ml free ciprofloxacin and equivalent concentration of Cip Arg-MSN. Statistical significance was calculated as p-value (**<0.001, ***<0.0001).

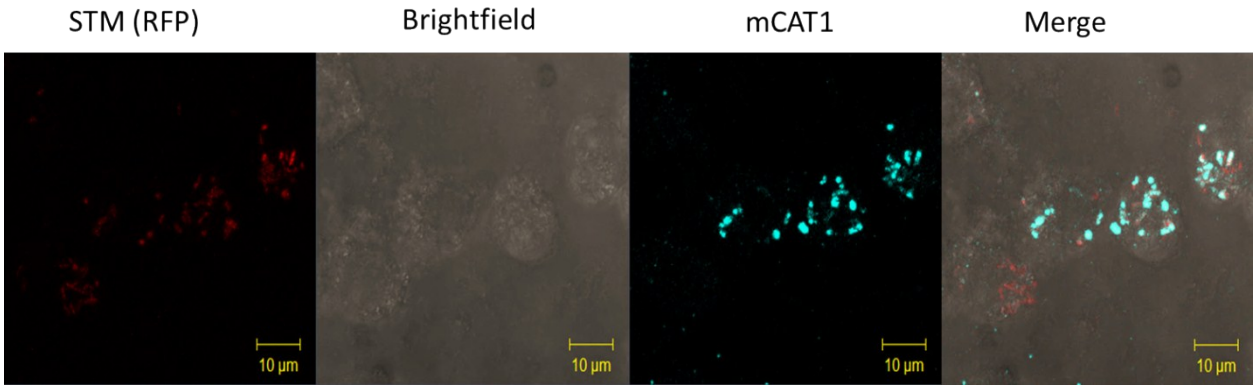

Figure S4: Co-localization of intracellular STM with mCAT1 transporter at 2 h post infection. The co-localization was studied by staining with anti mCAT1 antibody with Cy3 conjugated secondary antibody. The result indicates complete co-localization between mCAT1 and STM.

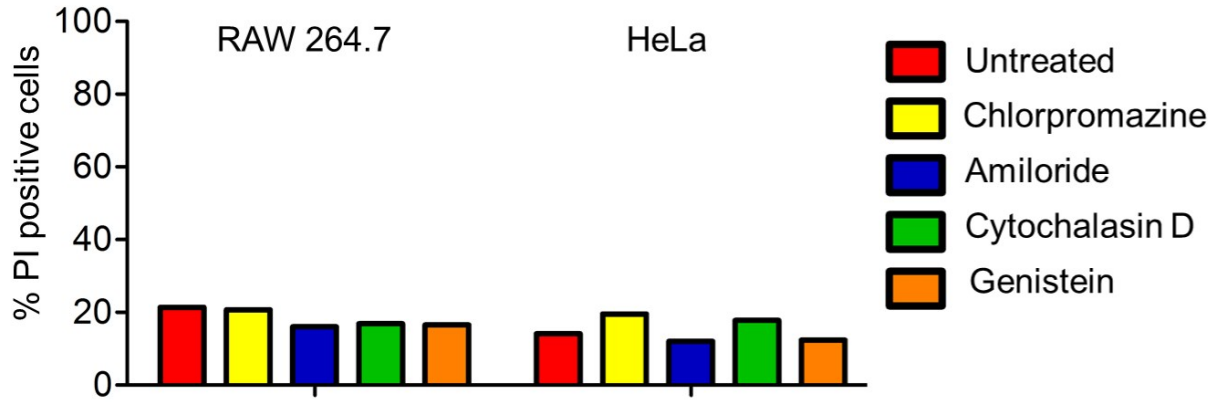

Figure S5: *In vitro* cytotoxicity of the pharmacological inhibitors. *In vitro* toxicity in RAW 264.7 and HeLa cells were studied after incubation for 2 h with the endocytosis inhibitors, Chlorpromazine 25 µM, Amiloride 5 mM, Cytochalasin D 4 µM and Genistein 100 µM. Chlorpromazine (CPM) blocks clathrin mediated endocytosis, amiloride inhibits macropinocytosis, genistein is known to block caveolae mediated endocytosis and cytochalasin D is known to inhibit actin dependent endocytosis. The toxicity was assessed by flow cytometry determination of percentage of cells positive for propidium iodide (PI).