Supplementary Information for:

Biologically triggered exploding protein based microcapsules for drug delivery

Krishna Radhakrishnan and Ashok M. Raichur

Department of Materials Engineering, Indian Institute of Science, Bangalore, 560012 India. Fax: +91 80 23600472; Tel: +91 80 22933238 E-mail: amr@materials.iisc.ernet.in

Materials

Protamine sulphate salt from salmon (PRM, Mw ~ 5.1kDa), heparin sodium salt from porcine intestinal mucosa (HEP, Mw~ 17 – 19 kDa), poly (styrene sulfonate) (PSS, Mw =70kDa), trypsin (Mw ~ 23.3 kDa), sodium carbonate, calcium chloride, ethylene diamine tetra-acetic acid (EDTA), dextran-TRITC (Mw ~65-85kDa), and Phosphate Buffer Saline (PBS) was purchased from Sigma Aldrich, India. Ciprofloxacin hydrochloride was a gift by Dr. Reddy's laboratories Ltd., India. All the chemicals were used without any further purification.

Synthesis of PSS doped $CaCO_3$ microparticles

In a typical experiment, 2 mg/mL PSS was suspended in 0.33 M $CaCl_2$ and kept under magnetic agitation for 30 minutes. To the above solution an equal volume of 0.33 M Na_2CO_3 was added rapidly at room temperature. This resulted in a white precipitate consisting of $CaCO_3$ microparticles. These particles were collected and washed by membrane filtration (with a cellulose filter having pore size of 0.45 μm). The as prepared particles were dried and stored until use.

Zeta potential measurements

The zeta potential measurements were carried out at various stages of microcapsule fabrication by using a Zetasizer Nanoseries NanoZS90 (Malvern Instruments, UK). The samples were diluted with an equal volume of pH adjusted water (pH = 6). Each zeta potential value is an average of 3 parallel measurements.

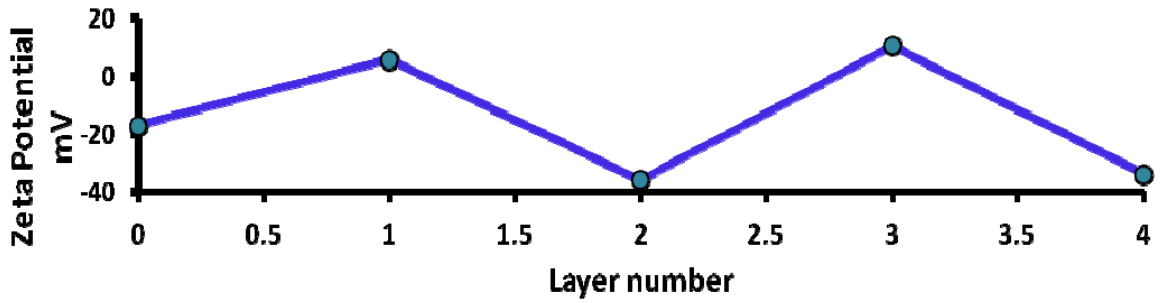

Fig.S1 Zeta potential measurements at various stages of microcapsule fabrication. The point 0 on X axis corresponds to the bare $CaCO_3$: PSS microparticles and the subsequent points 1, 2, 3 and 4 on X axis correspond to the subsequent layers adsorbed on to the microparticle template

Characterisation of microcapsules

The SEM images were obtained using a Scanning Electron Microscope (SEM) fitted with a field emission gun (FEI sirion, Eindhoven, Netherlands). The samples were placed on silica wafer, air dried and Au coated before analyzing with SEM. The images were obtained at 10kV with a working distance of 10 cm. Confocal Laser Scanning Microscopy (CLSM) images were acquired with a Zeiss LSM 510 META confocal scanning system (Zeiss, Germany) equipped with a 100× oil immersion objective with a numerical aperture of 1.4. Individual samples were placed on a cover glass and excited with a laser of wavelength 543nm. The EDX spectra of the microcapsules were taken before and after core dissolution.

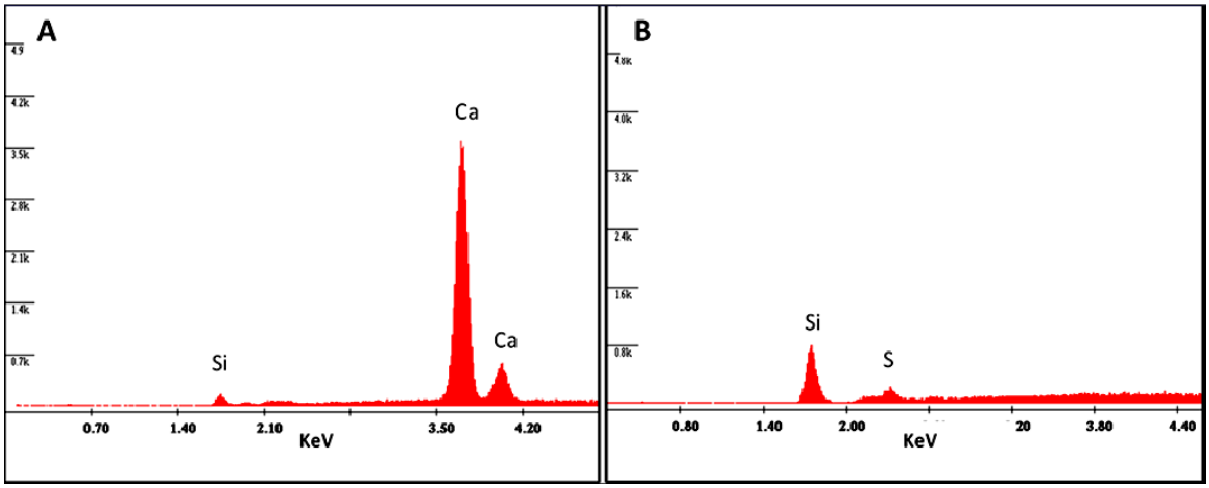

Fig.S2 EDX spectra of the microcapsules before (A) and after (B) core dissolution

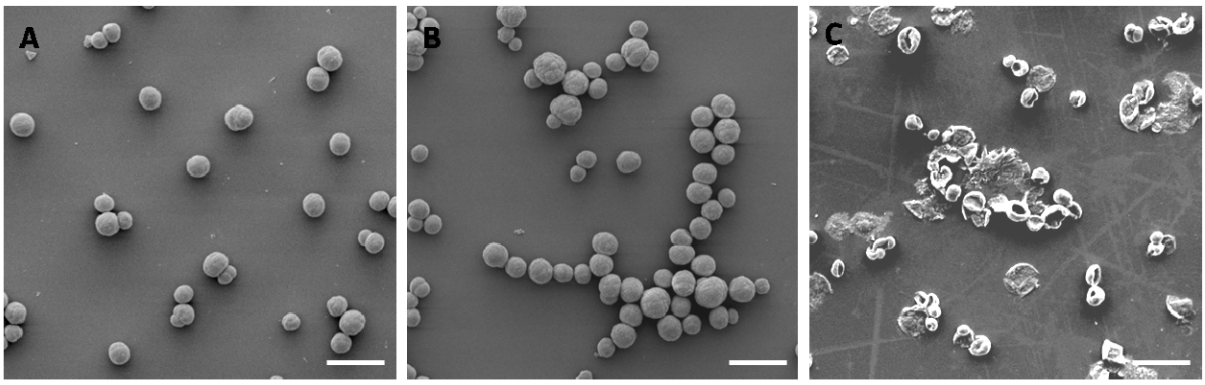

Fig. S3 Scanning electron microscopy (SEM) image of $CaCO_3$: PSS microparticle before (A) after LbL assembly (B) and hollow air dried PRM/HEP microcapsules (C). (The scale bar = 10µm)

Dextran-TRITC loading and release experiment

500µl of a suspension containing the hollow PRM/HEP microcapsules at pH 3 was taken in an eppendorf tube. 500µl of 1mg/ml dextran-TRITC was added to this suspension and incubated for 12 h while maintaining the acidic pH. Finally, the pH of this solution was adjusted to 7.4 using PBS to close the open pores of the microcapsules. In order to study the enzyme mediated release of dextran-TRITC from the loaded microcapsules, 500µl of the above suspension was added to a solution of trypsin in PBS buffer pH 7.4(final concentration of 0.04mM). The samples were then immediately viewed under confocal microscope.

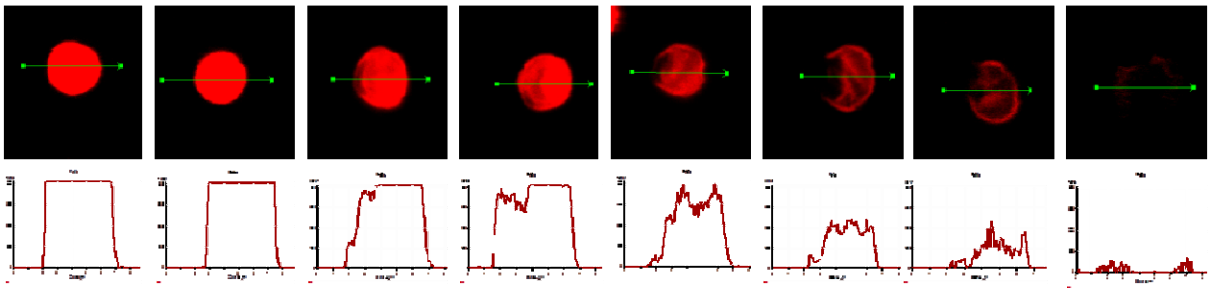

Fig.S4 Movie showing the kinetics of microcapsule explosion upon treatment with trypsin. The time interval between images is 2 mins

Evaluation of Drug loading efficiency using model drug ciprofloxacin hydrochloride

A suspension of hollow microcapsules ($1.05x10^7$ capsules/ml, pH3) was incubated with 1mg/ml ciprofloxacin hydrochloride. This mixture was kept at room temperature for 12 hours while maintaining the acidic pH. The open pores of the microcapsules were then closed by readjusting the pH to 7.4 using PBS buffer. The amount of the drug loaded into the microcapsule cavity was obtained from the difference in absorbance between the initial drug solution before loading and the supernatant obtained after centrifuging the loaded capsules.

The microcapsules exhibited a loading efficiency of 9.0058 x 10^{10} molecules per capsule, for the model drug ciprofloxacin hydrochloride. The absorbance was measured at 276 nm using ND-1000UV-Vis spectrophotometer (Nanodrop Technologies, USA) and the number of microcapsules was counted using a haemocytometer and optical microscope.