

Supporting Information

for

A Facile and Efficient Strategy to Encapsulate the Model Basic Protein Lysozyme into the Porous CaCO₃

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Section 1. Lysozyme standards.

The amount of lysozyme was quantified spectrophotometrically by BCA assays. The standard lysozyme solutions with concentration range of 25-1000 μg (0.1 M, pH 7, adjusted by NaOH solution) was mixed with BCA working reagent with equal volume (100 μl) into 96-well plates. The samples were incubated at 60 $^{\circ}\text{C}$ for 30 min. After cooling down to room temperature, the absorbance at 562 nm was tested. After replicating 3 times each sample, the average value was used. The standard curve was obtained by fitting the lysozyme concentration and absorbance intensity, as shown in Fig S1. And the fitted equation was listed in the inset of the Fig S1.

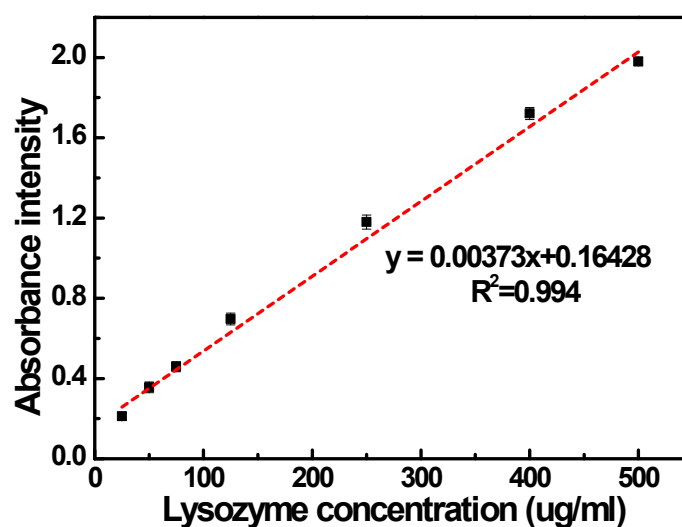


Figure. S1. The standard curve of lysozyme.

Section 2. *Micrococcus lysodeikticus* cells culture

2.1 Preparation of Luria-Bertani (LB) medium

LB liquid medium was prepared by dissolving 10 g of bacto-tryptone, 5 g of bacto-yeast extract and 10 g of NaCl in the bottle with 1 liter de-ionized water. LB solid

medium was prepared by dissolving 10 g of bacto-tryptone, 5 g of bacto-yeast extract, 20g of bacto-agar, and 10 g of NaCl in the bottle with 1 liter de-ionized water. The pH values of liquid and solid medium were adjusted to 7.4 with NaOH solution, then sterilized at 120 °C for 20 min, and stored at 4 °C after recover to room temperature.

2.2 Bacterial Growth Test

10 µL original bacteria suspension (purchased from purchased from China General Microbiological Culture Collection Center) was added into 50 ml of the liquid medium. After incubating for 18-24 h at 37 °C at 110 rpm within the incubator-shaker, the bacteria suspension lines were drawn onto the solid medium and cultivated them at 37 °C overnight. The single bacterial colony was picked up for repeating the bacteria suspension and bacteria lines growth. After 2 cycles, the bacteria suspension was collected as the recovery bacteria of *Micrococcus lysodeikticus cells* for next measurements.

The above bacteria suspension was added to the liquid medium with the ratio of 1:100 (v/v) and cultivated for 18-24 h at 37 °C at 110 rpm. The absorbance at 450 nm of bacteria suspensions was recorded at different time, and the dependence of the absorbance on the time was shown in Fig S2. It can be seen that the bacteria were in the exponential phase before 18 h, a steady stage was observed after that, and the degenerating stage appeared after the 26 h. The bacteria grown between 18~26 h were applied to test the bioactivity of lysozyme because of mature cytoderm and steady physical characteristics.

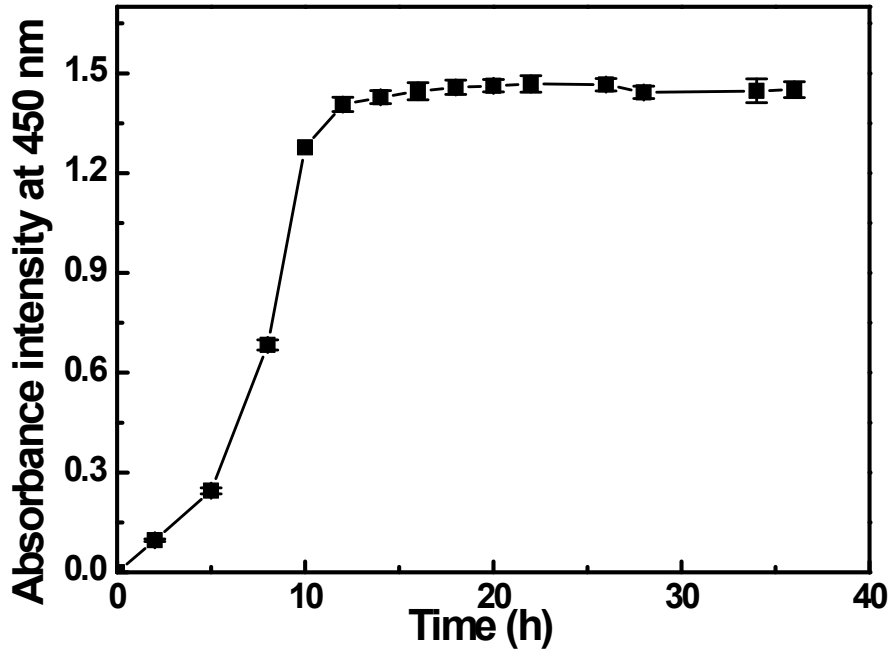


Figure S2. The dependence of the absorbance of *Micrococcus lysodeikticus* cells at 450 nm on the incubating.

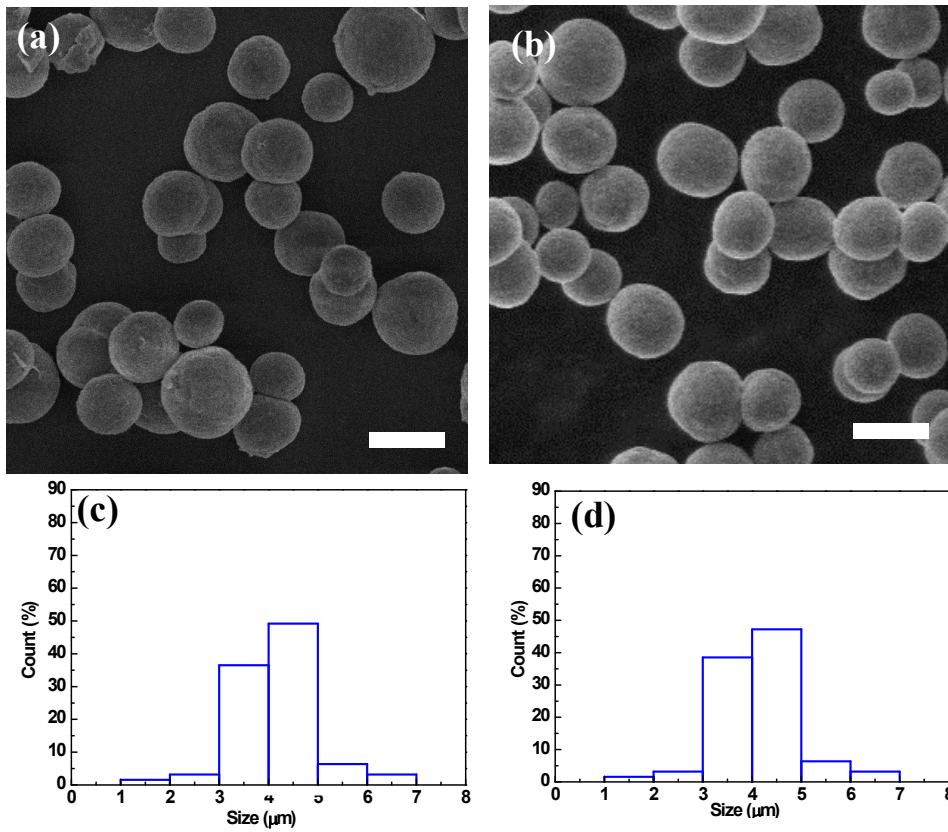


Figure S3. SEM images of a) Hep/CaCO₃ and b) pristine CaCO₃ particles; the size distribution of c) Hep/CaCO₃ and d) pristine CaCO₃ particles. The scale bars were 5 mm in a) and b).

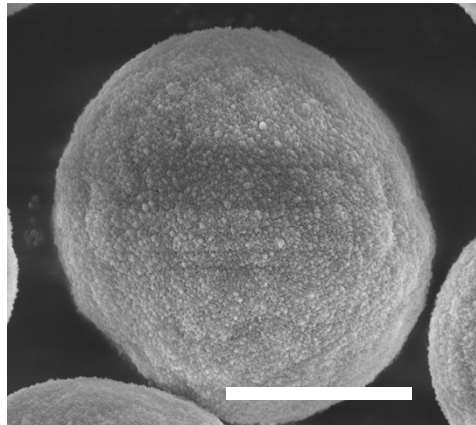


Figure S4. SEM image of the pristine CaCO₃ particles with loaded lysozyme by co-precipitation method. Scale bar 2 μm.

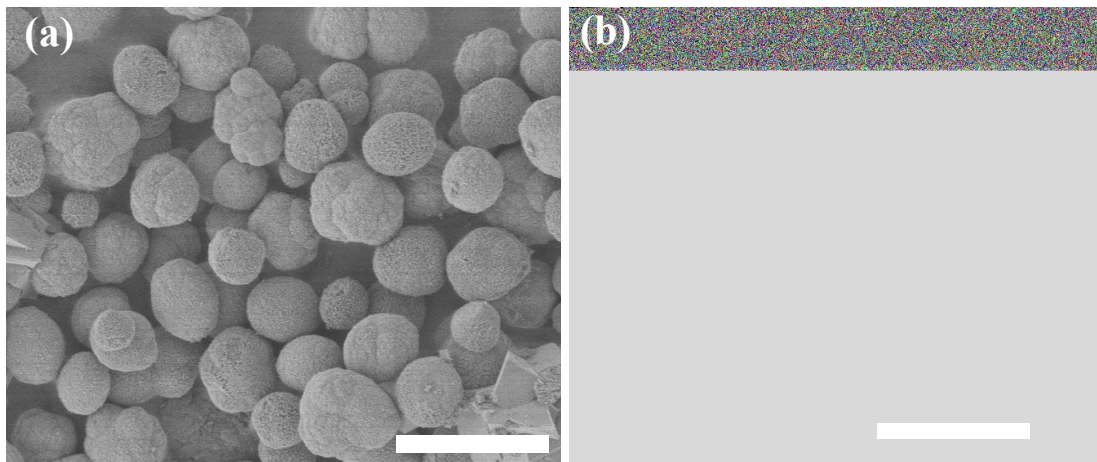


Figure S5. SEM image of (a) pristine CaCO₃ and (b) Hep/CaCO₃ particles loaded with lysozyme after 200 min incubation. Scale bar 10 μm

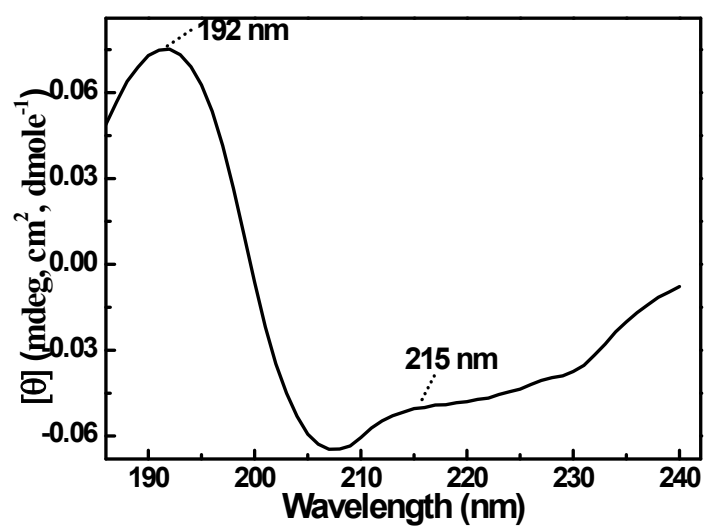


Figure S6. CD spectrum of lysozyme in LBL capsules.