Supporting Information

Protein-directed synthesis of ZIF-8 functionalized with polymer as core-shell drug coatings with antibacterial and anti-inflammatory properties

1. Experimental section

Chemicals: Pluronic P123, Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$), 2-methylimidazole (2-MeIm), Lysozyme, and Epigallocatechin gallate (CAS No. 989-51-5), were purchased from Sigma-Aldrich Inc. Bacterial cells *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. Methicillin resistant *S. aureus* (MRSA) (ATCC 33591), were obtained from ATCC (Rockville, Md). Mueller Hinton (MH) agar and broth were obtained from HiMedia (Mumbai, India). All of the other chemicals were used without any further purification.

Characterization: All the morphological analysis was done using a scanning electron microscope (SEM; JEOL JSM7600F) with a field emission source at 5 kV. The hydrodynamic size of nanocomposites in PBS was also determined by dynamic light scattering using a NanoSeries Zetasizer ZS 90 (Malvern Instruments Ltd, Malvern, UK). The surface chemical character of nanocomposites was detected in the wavelength range of 400-4000 cm^{-1} using Fourier transform infrared spectroscopy (FTIR) by Perkin Elmer-Spectrum 2, Waltham, MA, USA. X-ray diffraction (XRD) analysis of nanocomposites was determined using a Bruker D8 Discover XRD diffractometer in a 2θ range of 5–50°. The surface area and pore-volume distribution analysis were measured using Brunauer–Emmett–Teller (BET) surface area analyzer (Micromeritics, U.S.A.). Absorption spectral measurements were recorded at room temperature using Perkin Elmer Lambda UV/Vis spectrophotometer.

Lysozyme-directed synthesis of ZIF-8 (LZIF-8): In brief, 50 mg of the lysozyme (50 mL) were mixed with 4 mL methanol solution of 0.5 M $Zn(NO_3)_2.6H_2O$) and stirred for 15 min at room temperature. Subsequently, the 3.5 M of 2-MeIm dissolved in 40 mL methanol solution was quickly introduced into the above mixed solution and the mixture was reacted under stirring for another 15 min at room

temperature. Finally, the product (LZIF-8) was purified via centrifugation–wash cycles three times using methanol (7000 rpm, 10 min), and dried at room temperature.

Synthesis of POL@LZIF-8 nanocomposites: Pluronic P123 polymer was dissolved in methanol (10 wt%) at room temperature and stirred until a clear solution was obtained. A known concentration of synthesized LZIF-8 colloidal solution was dissolved in methanol and sonicated to prevent particulate aggregation. For preparation of the POL@LZIF-8 nanocomposites, 30 mg of LZIF-8 was mixed with of 10 mg polymer in 25 ml of methanol solution to control the MOF-polymer weight loading and then further stirred for 15 min at room temperature. Then, the product (POL@LZIF-8) were collected by centrifugation (7000 rpm, 10 min), and washed three times with methanol and finally, dried at room temperature for further use.

Drug Loading: A 5 mg amount of POL@LZIF-8 nanocomposites was dispersed in EGCG aqueous solution (2 mL, 0.5 mg/mL) and stirred for 24 h at room temperature and finally, the prepared drug-loaded nanocomposites were collected after centrifugation of the solution. To calculate the amount of drug loaded into the nanocomposites, the contents of original drug and the supernatant were measured by the UV/Vis estimations. The drug loading capacity of EGCG was calculated by the following equations.

$$Loading\ efficiency\ (\%) = \frac{Mass\ of\ original\ EGCG - Mass\ of\ EGCG\ in\ supernatant}{Mass\ of\ original\ EGCG} \times 100$$

***In vitro* drug release analysis:** The *in vitro* release rate of EGCG from POL@LZIF-8 nanocomposites were measured at physiological pH 7.4 in phosphate buffer solution (PBS). 5 mg of POL@LZIF-8 nanocomposite was dispersed in 10 mL of PBS solution and incubated at pH 7.4 in a shaking incubator (80 rpm). The amount of EGCG released was recorded as absorbances by a UV-Vis spectrometer (Perkin Elmer, USA) using a standard calibration curve at different time intervals.

Antibacterial activity: Minimum inhibitory concentrations (MIC): The antibacterial activity of EGCG-loaded POL@LZIF-8 nanocomposites were studied against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and MRSA (ATCC 33591). The bacterial culture was prepared by our previous

optimized method. The bacterial culture was grown to the stationary phase in MH broth (MHB) followed by their growth until mid-log phase was achieved to obtain turbidity, confirmed by an absorbance equivalent to 0.6 at a wavelength of 600 nm. Then a 1:200 dilution in MH broth was performed to obtain a final concentration of $5x10^5$ CFU/mL, and the diluted bacterial suspension (100 μL) was incubated with the serially diluted nanocomposites in the 96-well plate. Negative and positive growth controls were performed by adding only MHB or bacterial cells with MHB in the wells. At the end of the incubation time, MIC was determined as the lowest compound concentration at which no bacterial growth was observed.

Time-kill assay: Furthermore, for evaluation of the antibacterial activity, $5x10^5$ CFU/mL concentration of bacterial cells was inoculated with EGCG-loaded POL@LZIF-8 nanocomposites. Colony formation was observed at particular time points in the plates at 37°C.

ROS generation assay: Next, we determined the lipid peroxidation generation (ROS) in cell membranes of bacteria by EGCG-loaded POL@LZIF-8 nanocomposites. For this, different concentrations of nanocomposites (5-20 μg/mL) were incubated with *E. coli* and *S. aureus* (10^5 CFU) for 30 min and the lipid peroxidation was quantified by malonyldialdehyde (MDA)-intermediary product estimation using TBARS method. The formed MDA was measured spectrophotometrically at 530 nm.

Morphology of bacteria: The bacterial cells were incubated with the nanocomposites at 37°C for 4 h, followed by overnight fixation with 2.5% glutaraldehyde at 37°C, PBS washing and finally lyophilized. The morphologies of bacterial cells were imaged using a scanning electron microscope.

Cell membrane integrity assay: The membrane integrity of bacterial cells was studied using nucleic acids leakage assay. To quantify the nucleic acids leakage, control and nanocomposites-treated *E. coli* and *S. aureus* cell suspensions were centrifuged, re-dissolved in PBS (0.1M, pH 7.2) and then treated with or without the EGCG-loaded POL@LZIF-8 nanocomposites at 37°C with constant stirring. The samples were collected, centrifuged (8000 rpm, 5 min) and supernatant was collected at particular time

points. The nucleic acid concentration was measured spectrophotometrically at 260 nm. Bacterial cells without nanocomposites treatment served as control group.

Anti-inflammatory activity: Human leukemic cell line THP-1 (10^5 cells/well) were seeded into a 24 well plate and differentiated into macrophages. THP-1 cells were then treated with various concentrations of EGCG-loaded POL@LZIF-8 nanocomposites for 12 h with or without LPS (1 μg ml^{-1}). Cell-free supernatants were collected and analysed for IL-1β and IL-6 secretions using corresponding ELISA kits according to manufacturer's protocol.

Statistical analysis: The results are reported as mean ± standard deviation for n = 3 (unless otherwise stated). Statistical analysis was performed using GraphPad Instat, software version 3.0 (San Diego, CA). The values were analyzed by a one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test at a significance level of $p < 0.05$.

2. Supplementary Figures and Tables

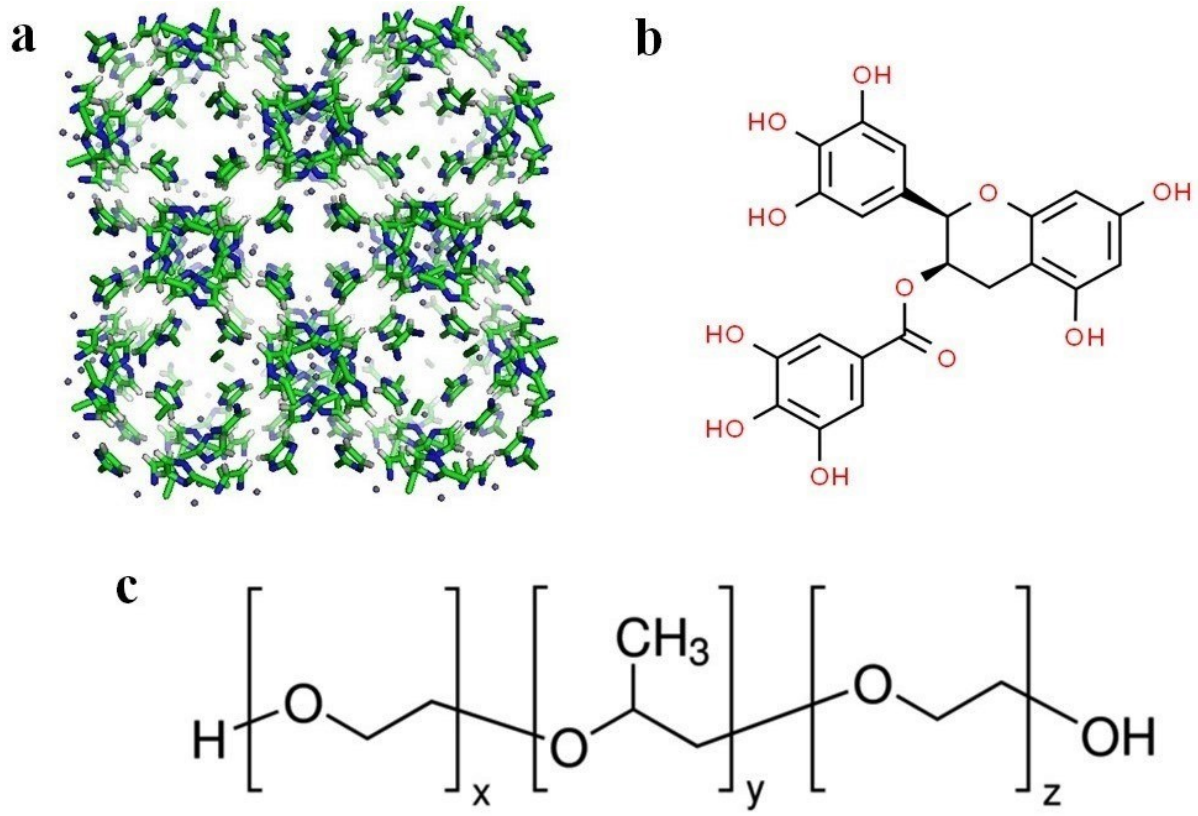

Fig. S1. Structure of (a) ZIF-8; (b) Epigallocatechin gallate and (c) PEG-PPG-PEG polymer.

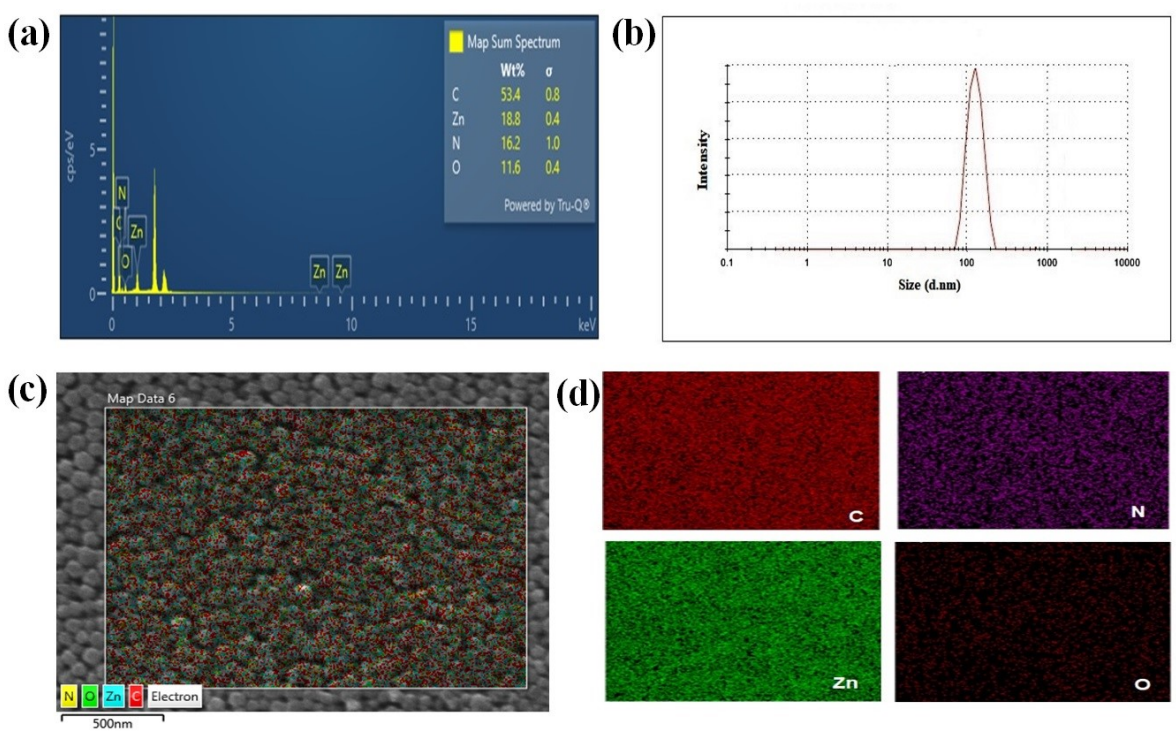

Fig. S2. (a) EDX analysis, (b) size distribution by DLS, (c) and (d) EDS elemental mapping images of POL@LZIF-8 nanocomposites