Supporting information

Self-assembly amphiphilic polysaccharide-based co-delivery system for egg white derived peptides and curcumin with oral bioavailability enhancement

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Methods

Transmission Electron Microscopy (TEM). TEM images were conducted with a Tecnai Spirit electron microscope (FEI, Netherlands). Each of sample solution was dropped onto a copper wire mesh and negatively stained by phosphotungstic acid in advance.

Atomic Force Microscopy (AFM). The AFM observation for samples was performed using a Veeco Nanoscope V Multimode 8 scanning probe microscope under the ambient conditions (25 °C, relative humidity of 25%). Prior to AFM scanning, 10 μL of each sample was dropped on the smooth mica sheets carefully and air-dried for 4 h.

X-ray Photoelectron Spectroscopy (XPS). The surface elemental composition of samples was detected by a Thermo ESCALAB 250Xi spectrometer (Waltham, MA) using an Al Kα X-ray excitation source. The high-resolution spectra were obtained at a 30.00 eV pass energy with a step size of 0.050 eV.

Fourier Transform Infrared Spectroscopy (FTIR). FTIR was performed to explore the interaction mechanism within the NPs. Different samples were freeze-dried in advance and then made into tablets at a specific mass ratio (sample:potassium bromide = 1:100). The corresponding spectra were recorded at a resolution of 4 cm⁻¹ over the 4000-400 cm⁻¹ range.

¹H NMR. Lyophilized samples were fully dissolved in deuterated dimethyl sulfoxide (DMSOd6) to reach a concentration of 5 mg/mL. Then, the ¹H NMR spectra was determined by a Bruker Advance III 500 MHz spectrometer (Billerica, MA). The chemical shifts were reported in ppm, respectively.

X-ray Diffraction (XRD). To better illustrate the co-encapsulation mechanism of EWDP and curcumin in NPs, freeze-dried samples were detected by an X-ray diffractometer (Bruker, Germany) with 40 kV accelerating voltage and 40 mA tube current. The 2θ angel range was set as 5-50° with a scanning rate of 0.24°/min.

Differential Scanning Calorimetry (DSC). DSC was performed to investigate the thermal properties and crystallinity of NPs. Samples were heated from 30 to 230 °C (10 °C/min) in the hermetically sealed aluminum pans with a nitrogen flow of 20 mL/min.

Cytotoxicity Assay Caco-2 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, 1% nonessential amino acid and 1% penicillin-
streptomycin. The cells (90 μL) were seeded in the 96-well plates with a density of 8000 cells per well, respectively. After overnight incubation, the cells were exposed to the samples (10 μL) diluted in phosphate buffered saline (PBS) with different EWDP/curcumin concentrations for 24 h. Afterwards, 20 μL MTS solution was loaded to each well for 2 h (37 °C, 5% CO₂), and then the UV absorbance at 490 nm was recorded by a microplate reader (BioTek, Winooski, VT) to calculate the corresponding cell viability. 
Fig. S1 The mass spectrum of EWDP (CYST). The corresponding b and y ions were shown in the spectrum.
Fig. S2 Zeta potential of native core-shell materials and solvent under various pH values (2.0-7.0).
Fig. S3 The size distribution of samples under different pH values (2.0-6.0). (a) HTCC-β-CD NPs. (b) HTCC-EWDP-β-CD-cur NPs.
Fig. S4 Images of samples under different pH values (2.0-7.0). (a) The simple mixture of EWDP and curcumin. (b) HTCC-β-CD NPs. (c) HTCC-EWDP-β-CD-cur NPs. The concentration of EWDP and curcumin for different samples was 0.5 mg/mL and 0.05 mg/mL.
Fig. S5 Schematic illustration of different protons distribution within the β-CD molecule.

Table S1 $^1$H NMR analysis of β-CD protons’ chemical shifts within the composite NPs after encapsulation

<table>
<thead>
<tr>
<th>Protons</th>
<th>$\Delta\delta_{\text{HTCC-β-CD}}$</th>
<th>$\Delta\delta_{\text{β-CD-cur}}$</th>
<th>$\Delta\delta_{\text{HTCC-β-CD-cur}}$</th>
<th>$\Delta\delta_{\text{HTCC-EWDP-β-CD-cur}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>-0.001</td>
<td>-0.003</td>
<td>-0.004</td>
<td>-0.020</td>
</tr>
<tr>
<td>H2</td>
<td>-0.001</td>
<td>-0.004</td>
<td>-0.004</td>
<td>-0.021</td>
</tr>
<tr>
<td>H3</td>
<td>0.000</td>
<td>-0.009</td>
<td>-0.009</td>
<td>-0.314</td>
</tr>
<tr>
<td>H4</td>
<td>0.003</td>
<td>0.000</td>
<td>-0.001</td>
<td>-0.002</td>
</tr>
<tr>
<td>H5</td>
<td>0.000</td>
<td>-0.006</td>
<td>-0.007</td>
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</tr>
<tr>
<td>H6</td>
<td>0.002</td>
<td>-0.005</td>
<td>-0.007</td>
<td>-0.020</td>
</tr>
</tbody>
</table>

$\Delta\delta = \delta_{\text{the corresponding NPs}} - \delta_{\text{β-CD}}$. 
Fig. S6 Cytotoxicity evaluation for different samples. All the samples showed no significant cytotoxicity on Caco-2 cell after incubation for 24 h (cell viability ≥ 90%).
References


