Electronic Supplementary Information for

Facile fabrication of shell crosslinked microcapsule by visible light induced graft polymerization for enzyme encapsulation

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Table of contents

Pages S2-S3. Materials and methods
Pages S4-S6. Supporting figures and table
Page S6. References
Materials and methods

Materials

O-dianisidine, horseradish peroxidase (HRP), GOD (> 100000 U/g), polyethyleneimine (PEI, $M_n = 60000$ Da) and poly(ethylene glycol) diacrylate (PEGDA, $M_n = 575$ Da) were purchased from Sigma-Aldrich (St. Louis, MO). Thiouanthe catechol-O, O-diacetic acid (TX-Ct) was synthesized according to reported method. Modified Bradford protein assay kit was purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Ethylenediamine tetraacetic acid (EDTA) was from aladdin (Shanghai, China). All other reagents were obtained from Beijing Chemical Works (Beijing, China).

Preparation of GOD entrapped CaCO$_3$ microparticles

First, GOD (7.5 mg) was dissolved into 5 mL of 0.3 M CaCl$_2$ solution and stirred for 5 min. Then 5 mL of 0.3 M Na$_2$CO$_3$ solution was quickly added to the GOD solution and stirred vigorously for 5 min. The precipitate was collected by centrifugation using a high speed refrigerated micro-centrifuge (D3204R, Rotor type of AS24-2, Scilogex LLC., Rocky Hill, Connecticut, USA) at $1520 \times g$ (4000 rpm) at $4^\circ C$ and washed for 3 times with deionized water.

Preparation of GOD encapsulated microcapsules

0.6 g GOD loaded CaCO$_3$ microparticles was added to 50 mL of 1 mg/mL PEI solution (containing 0.5 M NaCl, pH was adjusted by HCl to 7.0) and stirred for 15 min. The pH value was determined by digital pH meter (FE20K, Mettler-Toledo, Switzerland) using a combination glass electrode, which was calibrated by standard pH 4.0 and pH 6.8 buffers. Then the CaCO$_3$ microparticles absorbed with PEI were collected by centrifugation at $1520 \times g$ (4000 rpm) at $4^\circ C$ and washed with phosphate buffered saline (PBS, pH 7.0) for 3 times. The obtained microparticles were added into 50 mL of 0.4 mg/mL TX-Ct solution (containing 0.5 M NaCl, pH 7.0) and stirred for 15 min. After centrifugation at $1520 \times g$ (4000 rpm) at $4^\circ C$ and washed with PBS (pH 7.0) for 3 times, the microparticles absorbed with PEI and TX-Ct were dispersed in 50 mL of 10 wt%, PEGDA aqueous solution (pH 7.0) and bubbled with nitrogen for 5 min. Then the system was irradiated by a LED lamp ($\lambda = 420$ nm, 6000 $\mu W/cm^2$, 5 cm from sample) for 1.5 h. The microparticles with poly(PEGDA) shell were collected by centrifugation at $1520 \times g$ (4000 rpm) at $4^\circ C$ and washed for 3 times with deionized water. To remove the CaCO$_3$ core, microparticles were added to 50 mL of 0.2 mol/L EDTA solution (pH was also adjusted by NaOH to 7.0) for 15 min. The particles were centrifuged at 13683 $\times g$ (12000 rpm) at $4^\circ C$, collected and washed in EDTA solution by the same procedure for another two times. Finally, the GOD encapsulated microcapsules were obtained by centrifugation at 13683 $\times g$ (12000 rpm) at $4^\circ C$ after washing with deionized water for 3 times.

Determination of immobilization efficiency and loading amount of GOD

The un-immobilized GOD in the supernate was quantified using the Bradford method. The immobilization efficiency and loading amount of GOD were calculated by the following equations:

Immobilization efficiency of GOD in CaCO$_3$ (%) = $(W_i - W_1) / W_i \times 100$

Load amount (mg/g) = $(W_e - W_2) / W$

Immobilization efficiency of GOD in microcapsule (%) = $(W_e - W_2) / W_e \times 100$

Where $W_i$ is the initial mass (mg) of added GOD, and $W_1$ (after co-precipitation) and $W_2$ (after removing CaCO$_3$ core) is the mass (mg) of GOD in supernatant, $W$ is the mass (g) of CaCO$_3$ microparticles loaded with GOD, and $W_e$ is the mass (mg) of GOD loaded in the CaCO$_3$ particles before graft polymerization.
**GOD activity assay**

The activity of GOD was determined by using o-dianisidine as the chromogenic agent (Scheme S1). Typically, 0.50 mL of glucose solution (0.1 g/mL), 0.1 mL of HRP solution (60 U/mL) and 2.4 mL of a 66 mg/L o-dianisidine solution in 0.1 M PBS were mixed in a cuvette at 25 °C. Then 0.1 mL of free enzyme solution or immobilized enzyme solution (0.1 U/mL) was added and the absorbance at 436 nm (molar extinction coefficient = $4.7 \times 10^3$ L/(mol·cm)) was measured every 1 min for 4 min until the increase in the absorbance per minute reached a stable value. Enzyme activity is defined as the amount of GOD that produces 1 μmol of hydrogen peroxide per minute under the conditions described above.

![Scheme S1. Principle of activity detection of GOD.](image)

**Reusability assay**

The activity of immobilized GOD was determined by same procedure described above. After each cycle of usage, the microcapsules were recovered by centrifugation at $13683 \times g$ ($12000 \text{ rpm}$) at 4 °C and cleaned with deionized water for three times then started next cycle. All the results were the average of three independent experiments.

**Characterization**

The morphology of the microparticles was observed by a field emission scanning electron microscopy (SEM; JSM-7500F, JEOL Ltd., Tokyo, Japan). The internal structure and thickness of the immobilized GOD was determined by biological transmission electron microscopy (TEM; JEM-1200EX, JEOL Ltd., Tokyo, Japan). The size and zeta potential of microparticles were measured by dynamic light scattering (DLS; ZetaPALS; Brookhaven Instruments Corp., Holtsville, NY, USA). The chemical composition and weight content of GOD encapsulated microparticles was characterized by Fourier transform infrared (FT-IR) spectroscopy using a Nexus 670 spectrometer (Thermo Nicolet Corp., Madison, WI, USA), thermal gravimetric analysis (TGA; Mettler Toledo, Shanghai, China) and X-ray photoelectron spectroscopy (XPS; Thermo ESCALAB 250XI, Thermo Fisher Scientific, USA). The UV–vis absorbance was recorded by a Hitachi U-3900H spectrophotometer. The fluorescence images were recorded on a confocal laser scanning microscope (CLSM, Leica SP8 from Leica, Germany).
Supporting figures

**Fig. S1.** SEM images of GOD entrapped CaCO₃ microparticles absorbed with 1 mg/mL (a, b) and 3 mg/mL (c, d) of PEI. (e) The adsorption amount of PEI with the change of PEI concentration. Values represents the mean ± SD of three independent experiments. The concentration of PEI was determined by colorimetry using Cu²⁺ as chromogenic agent.² Briefly, five PEI standard solutions with concentration of 0.02-0.2 μg/mL were prepared and the pH was adjusted by HCl to 7.0. One milliliters of each standard solution were added to 1 mL of a copper (II) sulphate solution (0.145 mg/mL) in 0.1 M acetate buffer at pH 5.4 at 20 °C. Upon addition of copper (II) ions, PEI forms a dark blue cuprammonium complex that can be detected by UV-vis spectrophotometry. The absorbance value of each solution was recorded at 285 nm (molar extinction coefficient = 4.85 × 10⁵ L/(mol·cm)) against a reagent blank (copper (II) sulphate solution). A calibration curve was obtained plotting absorbance versus concentration. The concentration of unabsorbed PEI in solution was determined by similar procedure after being diluted appropriately. The absorbed amount of PEI is the weight difference between used PEI and unabsorbed PEI.

**Fig. S2.** (a) The adsorption amount of TX-Ct with the change of TX-Ct concentration, (b) SEM images of GOD entrapped CaCO₃ microparticles absorbed with PEI and TX-Ct. The concentration of TX-Ct was determined by measured the absorbance of the solution at 390 nm (molar extinction coefficient = 3.66 × 10⁴ L/(mol·cm)). Values represents the mean ± SD of three independent experiments.
**Fig. S3.** XPS N 1s spectrum of CaCO$_3$.

**Fig. S4.** (a) The average size of CaCO$_3$ microparticles prepared in the presence of different concentrations of GOD, (b) The immobilization efficiency and loading amount of GOD in CaCO$_3$ microparticles at different concentration of GOD, and values represents the mean ± SD ($n = 3$).

**Fig. S5.** (a) FTIR spectra of GOD entrapped CaCO$_3$ microparticles grafted with poly(PEGDA) shell and GOD encapsulated microcapsules after removing the CaCO$_3$ template. (b) TGA curves of pure CaCO$_3$ (i), GOD (ii), GOD entrapped CaCO$_3$ (iii), GOD entrapped CaCO$_3$ microparticles grafted with poly(PEGDA) shell (iv) and GOD encapsulated microcapsules (v).

For the GOD entrapped CaCO$_3$ microparticles grafted with poly(PEGDA) shell (Fig. S6a), the characteristic absorption peak of vaterite CaCO$_3$ appears at 745 cm$^{-1}$. The peaks at 1736 cm$^{-1}$ was assigned to the vibration of ester carbonyl group of poly(PEGDA). After the CaCO$_3$
template was removed, strong characteristic amide absorption bands of enzymes at 1650 cm⁻¹ and 1545 cm⁻¹ appeared while absorption peaks belong to CaCO₃ disappeared.

**Table S1.** Element composition of pure CaCO₃, GOD entrapped CaCO₃ microparticles grafted with poly(PEGDA) shell (GOD@CaCO₃@poly(PEGDA)), and GOD encapsulated microcapsules (GOD@poly(PEGDA)) detected by SEM-EDAX analysis.

<table>
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<th>Element</th>
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**References**