Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Material

Decorating Individual Living Cell with a Thickness-Controllable Shell by Cytocompatible Surface Initiated Graft Polymerization

Guan Wang, Kai Zhang, Yindian Wang, Changwen Zhao, Bin He, Yuhong Ma and Wantai Yang**

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Experimental Section

Materials

Poly(ethyleneimine) (PEI, average M_n: ~60,000 by GPC, average M_w: ~750,000 by LS, 50 wt% in H_2O, Aldrich), poly(ethylene glycol) diacrylate (PEGDA, M_w = 575 g/mol, and M_w = 3400 g/mol, Aldrich), glycerol (Sigma-Aldrich), lyticase (≥2000 units/mg protein, from arthrobacter luteus, Sigma), fluorescein diacetate (FDA, Thermo Fisher Scientific), Tris-EDTA buffer solution (TE buffer, 1×TE, pH 7.4, SECOMA), thiosalicylic acid (97%, Aldrich), and catechol-*O*,*O'*-diacetic acid (97%, Alfa-Aesar) SYTO™ 9 Green Fluorescent Nucleic Acid Stain (SYTO 9, Thermo Fisher) were used without any purification. Hydrochloric acid (HCl, 35%), ulfuric acid (H_2SO_4, 98%), and glutaraldehyde (25%) were purchased from Beijing Chemical Works and used as received.

Synthesis of thioxanthone catechol-*O*, *O'*-diacetic acid (TX-Ct)

The procedures to synthesize TX-Ct was according to Karasu's work.[1] In brief, 0.32 g thiosalicylic acid was slowly added and dissolved in 15 mL sulfuric acid in an ice bath with a string for 5 min to ensure thorough mixing. Then, 1.35 g catechol-*O*,*O'*-diacetic acid was slowly added to the mixture in 30 min. The final mixture was stirred at room temperature for 3 days. Thioxanthone catechol-*O*,*O'*-diacetic acid (TX-Ct) was obtained by pouring the mixture into cold water carefully as the yellow product precipitated. TX-Ct was purified by water and the solid obtained by freeze-drying.

Poly(PEGDA) grafted from the surface of living yeast cell

Saccharomyces cerevisiae yeast cells were suspended in the yeast extract peptone dextrose (YPD) broth and cultured in a shaking incubator at 30 ^oC for 48 h. For PEI coating,[2] 30 mL of the yeast cells suspension was added into a 100 mL centrifuge tube, and the yeast cells were washed by 0.15 M NaCl for 3 times with a centrifugation of 3000 rpm for 3 min. The washed yeast cells were immersed in 0.5 mg/mL PEI solution (pH 5.0, adjusted by HCl) for 10 min, and washed by 0.01 M citrate buffer solution (pH 5.0) for once. After that, the PEI-coated yeast cells were mixed with 5 g PEGDA, 50 mL 0.01 M citrate buffer solution (pH 5.0), and 0.01 g TX-Ct. Then, the mixture was irradiated by visible light (λ=420, 6000 mW/cm^2) for 1 h under atmospheric conditions with magnetic stirring. Poly(PEGDA)-coated yeast cells were obtained by a series of washing procedures by deionized water for 3 times.

Poly(PEGDA) grafted from the surface of living aureus cell

Staphylococcus aureus cells was used as received. For the graft polymerization, *aureus* cells was washed by 0.01M PBS buffer (pH 7.2), immersed in 0.5 mg/mL PEI solution (pH 7.2, adjusted by HCl) for 10 min, and washed by 0.01 M PBS buffer (pH 7.2) for once. After that, the PEI-coated yeast cells were mixed with 1 g $PEGDA_{3400}$ (M_w=3400), 10 mL 0.01 M PBS buffer (pH 7.2), and 0.002 g TX-Ct. Then, the mixture was irradiated by visible light (λ=420, 6000 mW/cm^2) for 1 h under atmospheric

conditions with magnetic stirring. Poly(PEGDA)-coated yeast cells were obtained by a series of washing procedures by deionized water for 3 times.

Viability

The viability of yeast cells was measured by FDA staining. Because of FDA was poorly soluble in water, FDA was firstly dissolved in acetone to prepare 1 mg/mL FDA-acetone solution.[2] Then, 2 μL of the solution was mixed with the native or coated yeast cell suspension (0.1 M phosphate buffer solution, PBS, pH 6.5) and the mixture was incubated for 30 min at room temperature with a stirring in dark. The stained yeast cells were washed by 0.1 M PBS for 5 times for viability tests.

The viability of aureus cells was measured by SYTO 9 staining. 10 μL of SYTO 9 was added into 10 μL of aureus cells suspension, and incubated for 10 min at room temperature. The living aureus cells could be dyed green and observed by CLSM.

Cell-division and cell-lysis tests

Cell-division test was conducted by yeast cells cultured in a shaking incubator at 30 ^{o}C. First of all, the initial optical density of native or coated yeast cell was diluted to 0.01 of the absorbance at 600 nm in 0.1 M PBS (pH 6.5), and 50 μL of the diluted yeast cells suspension was added into the 100 mL YPD broth. Then, 1 mL of the broth was picked at the scheduled time with a gradient of 4 h, and the optical density of yeast cells suspension was measured at 600 nm.

The optical density of native of coated yeast cells was adjusted to 0.8 of the absorbance at 600 nm in the TE buffer (pH 7.4) for cell-lysis test. Lyticase solution was prepared by dissolving lyticase in the mixture of 500 μL glycerol and 500 μL TE buffer. For cross-linked lyticase solution, glutaraldehyde was employed as the cross-linker, and 10 μL glutaraldehyde (25%) was added into 100 μL lyticase solution for cross-linking at 4 ^{o}C for 0.5 h. The 50 μL of lyticase solution or 100μL of cross-linked lyticase solution was added into the yeast cells suspension (OD_{600}=0.8) at 37 ^{o}C, and the optical density of yeast cell suspension was determined every hours.

Instruments

Dynamic Light Scattering and Zeta Potential (Brookhaven Instruments Corp., USA) was used to detect the the zeta potential of the yeast cells. Scanning electron microscopy (SEM, JSM-7500F from JEOL Japan Electronics Co., Ltd, Japan) was used to determine the morphology of the yeast cells. The thickness of the poly(PEGDA) shell was measured by transmission electron microscopy (TEM, H-7650 from HITACHI, Japan). Fourier transform infrared spectroscopy (FT-IR) spectra of the yeast cells were recorded on a Nicolet NEXUS 670 spectrometer (Thermo Nicolet Co., USA). The elementary composition was probed by XPS (ESCALAB 250 from Thermo Fisher Scientific Co., USA) with a monochromator. The UV-vis Spectrophotometer (H3900, HITACHI, Japan) was used to determine the cell density at 600 nm. The fluorescence images were recorded on a confocal laser scanning microscope (CLSM, Leica SP8 from Leica, Germany) at an excitation

wavelength of 490 nm. Flow cytometer (MoFlo XDP from Beckman Coulter, USA) was used to determine the viability of yeast cells.

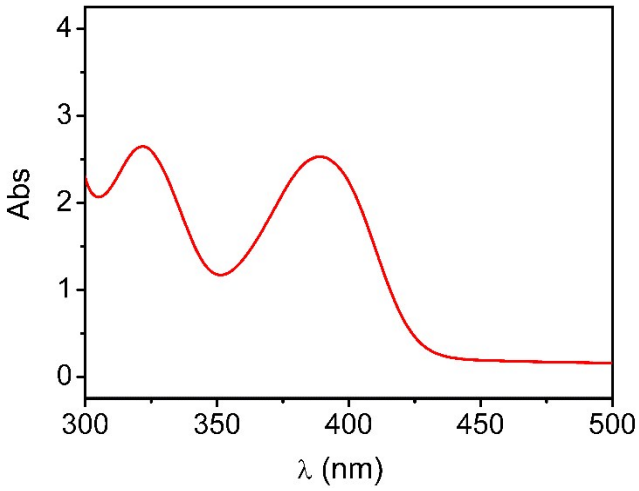

Fig. S1 UV-visible absorption spectrum of thioxanthone catechol-O,O'-diacetic acid (TX-Ct) dissolved in water. TX-Ct shows a broad absorption from 350-425 nm, which suggests that TX-Ct can be excited by visible light.

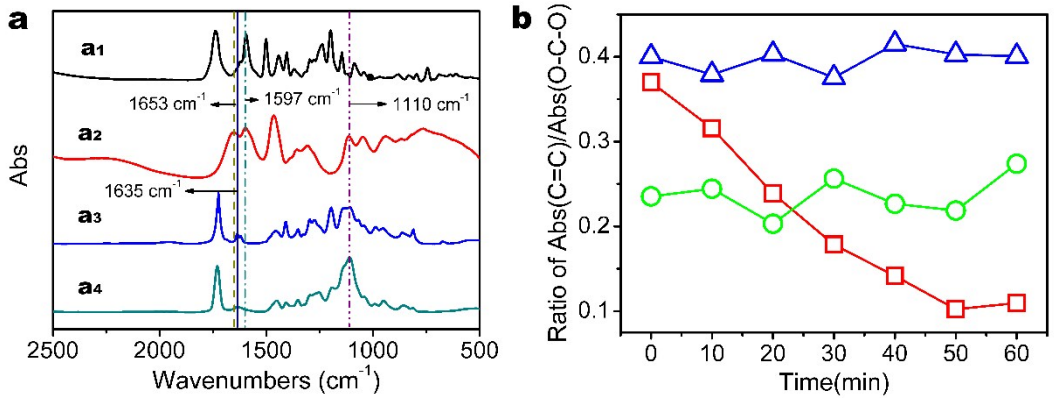

Fig. S2 (a) FTIR spectra of (a_1) TX-Ct, (a_2) PEI, (a_3) PEGDA, (a_4) poly(PEGDA) that initiated by TX-Ct and PEI after 60 min of polymerization. The peaks of 1653 cm^{-1}, 1635 cm^{-1}, 1597 cm^{-1}, and 1110 cm^{-1} represented for the absorption of primary amine of PEI, double bond of PEGDA, secondary amine of PEI, and ether bond of PEGDA, respectively. (b) The conversion of PEGDA was determined by calculating Abs(C=C)/Abs(O-C-O) obtained from FTIR, and the results were presented for the solution polymerizations with both PEI and TX-Ct added (**Reaction A**, ❍), only PEI added (**Reaction B**, ❒), and only TX-Ct added (**Reaction C**, ☌) versus irradiation time. Due to the absorption of primary amine and secondary amine at 1635 cm^{-1} and 1597 cm^{-1}, respectively, the original ratio of Abs(C=C)/Abs(O-C-O) of **Reaction A** and **B** was exceed **Reaction C**. After 50 min, all of the PEGDA was polymerized. It was found that the conversion of PEGDA exhibited an excellent linear relation with the polymerization time only in the presence of PEI and TX-Ct together. This result further demonstrated the validity of the initiating mechanism that was excited TX-Ct abstracting hydrogen from PEI.

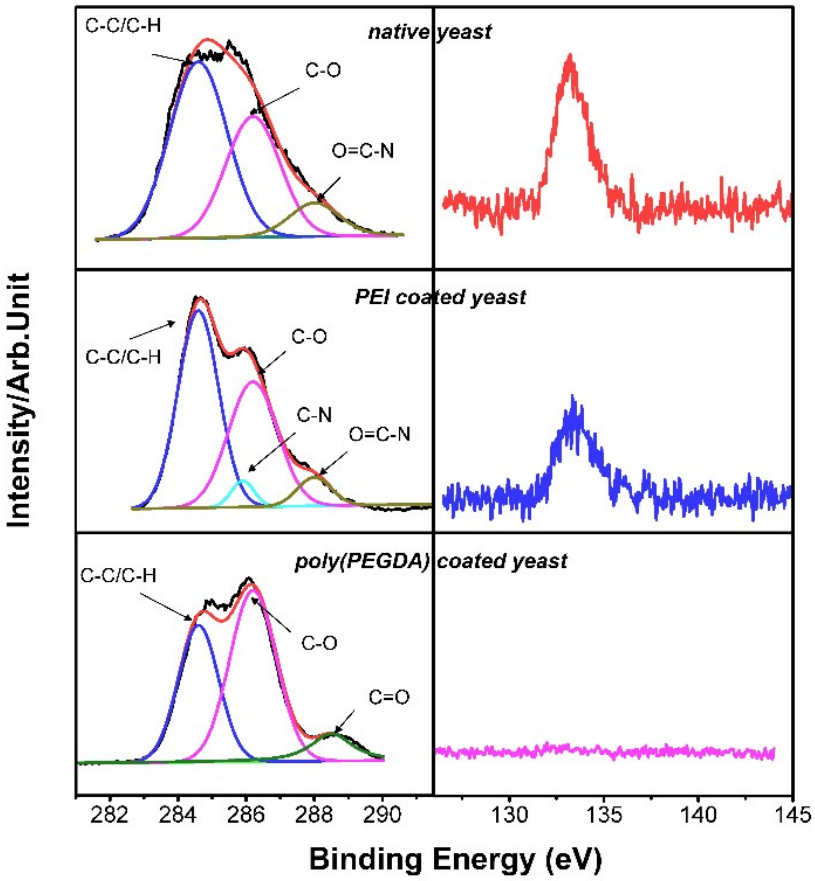

Fig. S3 XPS C 1s and P 2p core-level spectra of the native yeast cells, PEI-coated yeast cells, and poly(PEGDA)-coated yeast cells. The disappearance of the peak of O=C-N in C 1s core-level spectra and the P2p peak P 2p core-level spectra after formation of PEG shell confirmed that the poly(PEGDA) was successfully coated on surface of yeast cells.

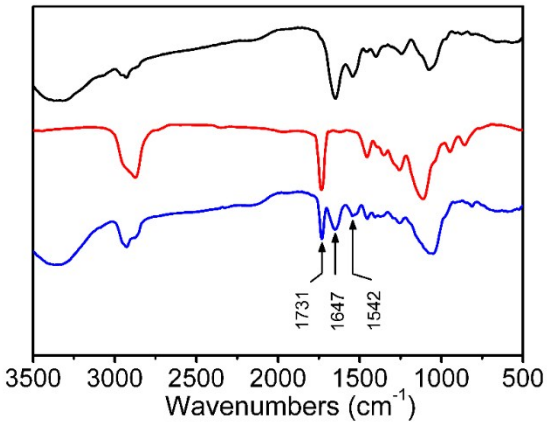

Fig. S4 FTIR spectra of native yeast cells (in black), pure poly(PEGDA) (in red), and yeast cells encapsulated by poly(PEGDA) (in blue). The peaks of 1731 cm^{-1}, 1647 cm^{-1}, and 1542 cm^{-1} represented for the absorption of C=O of PEGDA, amine I of yeast cells, and amine II of yeast cells, respectively. The appearance of the peak of C=O in the

FTIR spectra of the encapsulated yeast cells provided an evidence to the successful encapsulation.

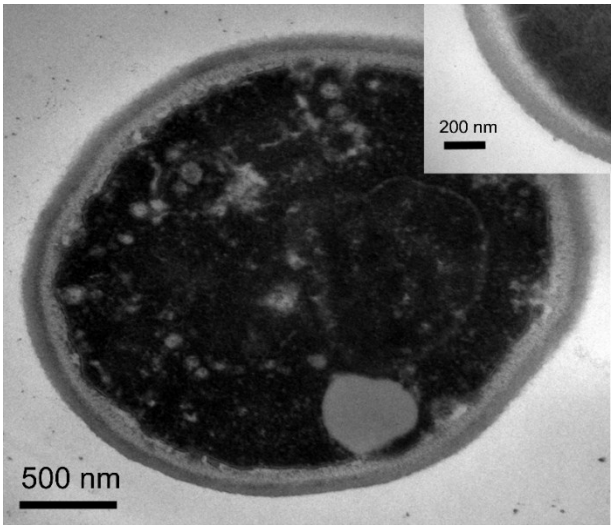

Fig. S5. TEM image of the native yeast cell with a magnification inserted.

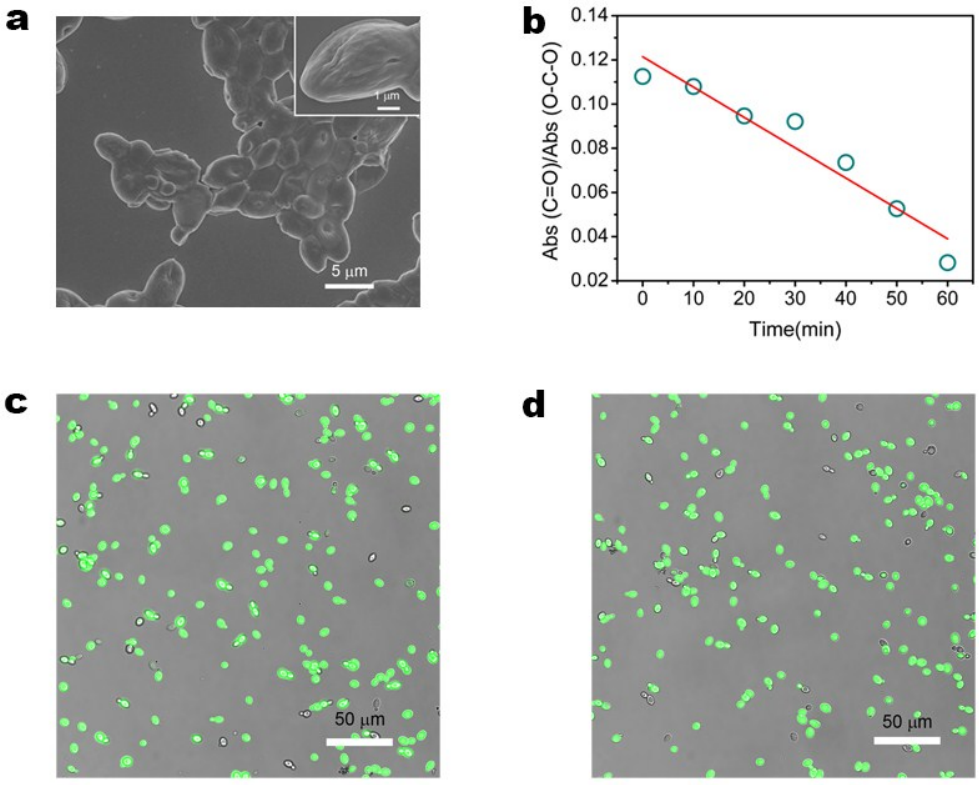

Fig. S6 (a) SEM image of poly($PEGDA_{3400}$) coated yeast cells. (b) The conversion of $PEGDA_{3400}$ was determined by calculating Abs(C=C)/Abs(O-C-O) obtained from FTIR, which proved that the thickness of the shell synthesized by $PEGDA_{3400}$ should be also controlled through adjusting polymerization time. CLSM images of (c) native yeast cells and (d) coated yeast cells.

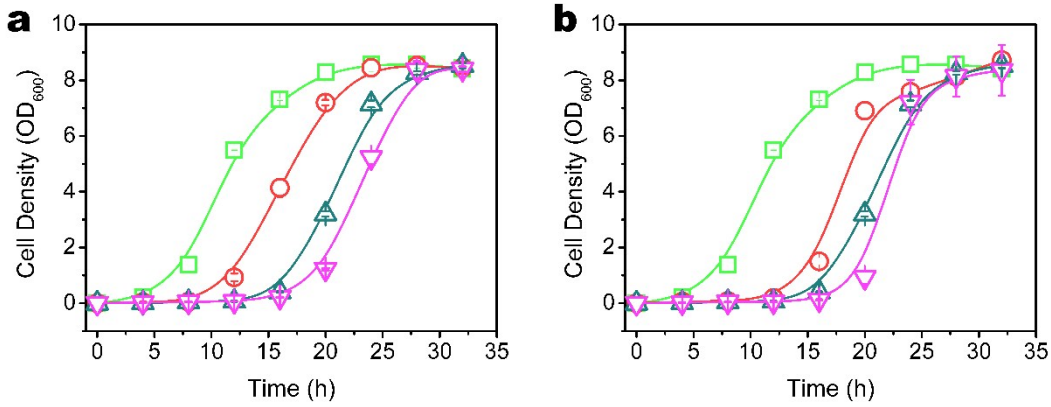

Fig. S7. (a) Cell growth curves of (❍) native and coated yeast cells with different monomer concentrations in graft polymerization for (ᔓ) 5 wt%, (❒)10 wt%, and (⧫)15 wt% monomer concentrations. (b) Cell growth curves of (❍) native and coated yeast cells with different irradiation intensities (λ=420 nm) in graft polymerization for (ᔓ) 3000 μW/cm^2, (❒) 6000 μW/cm^2, and (⧫) 9000 μW/cm^2 in the presence of lyticase. Optical density was measured at 600 nm (OD_{600}).

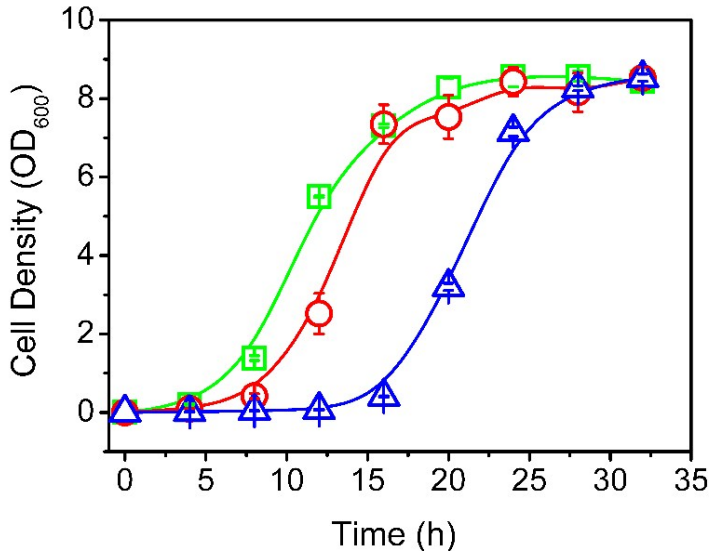

Fig. S8. Cell growth curves of (●) native yeast cells and coated yeast cells obtained from the graft polymerization (❒) with or (ᔓ) without the involvement of PEI. The optical density was measured using absorbance measurements at 600 nm (OD_{600}).

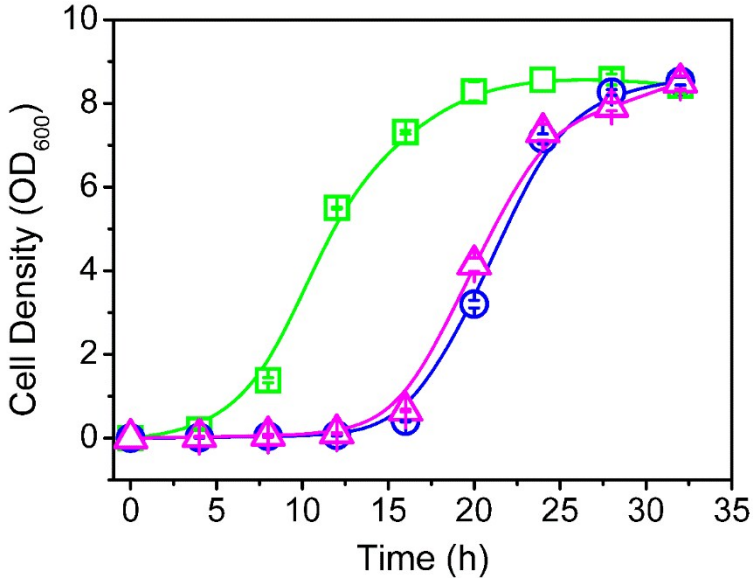

Fig. S9 Growth curves of (●) native and coated yeast cells obtained from the graft polymerization of PEGDA conducted in (ᔓ) air atmosphere and (❒) nitrogen atmosphere.

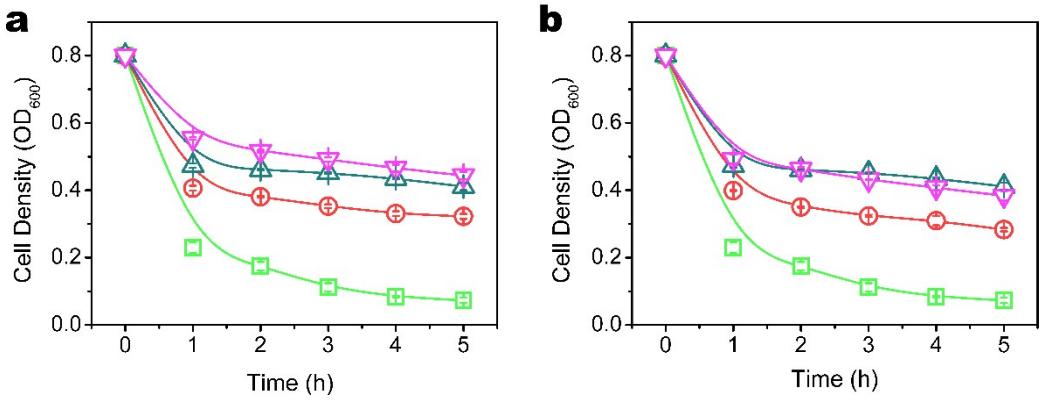

Fig. S10. (a) Survival curves of (●) native and coated yeast cells with different monomer concentrations in the graft polymerization for (ᔓ) 5 wt%, (❒)10 wt%, and (♦)15 wt% monomer concentrations. (b) Survival curves of (●) native and coated yeast cells with different irradiation intensities (λ=420 nm) in the graft polymerization for (ᔓ) 3000 μW/cm^2, (❒)6000 μW/cm^2, and (♦)9000 μW/cm^2 in the presence of lyticase. Optical density was measured at 600 nm (OD_{600}).

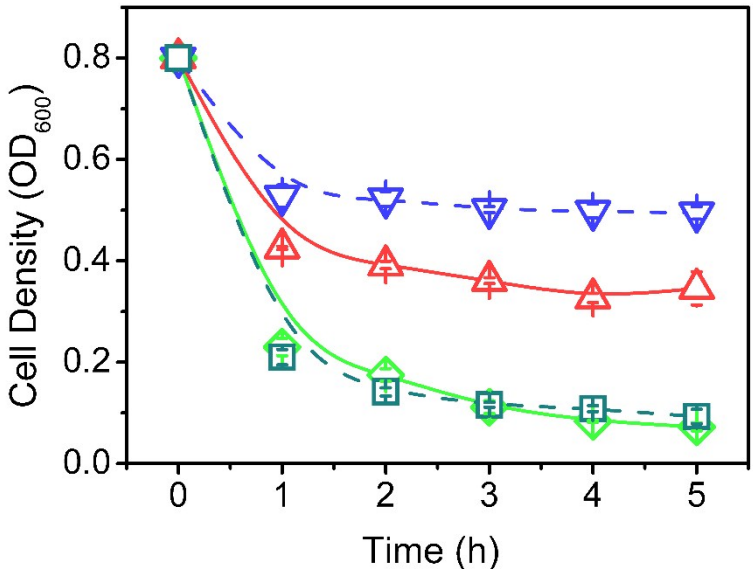

Fig. S11 Survival curves of (🕑) native and (❒) coated yeast cells of the graft polymerization of PEGDA in the presence of native lyticase. Survival curves of (●) native and (♦) poly(PEGDA)-coated yeast cells in the presence of cross-linked lyticase. The optical density was measured using absorbance measurements at 600 nm (OD_{600}).

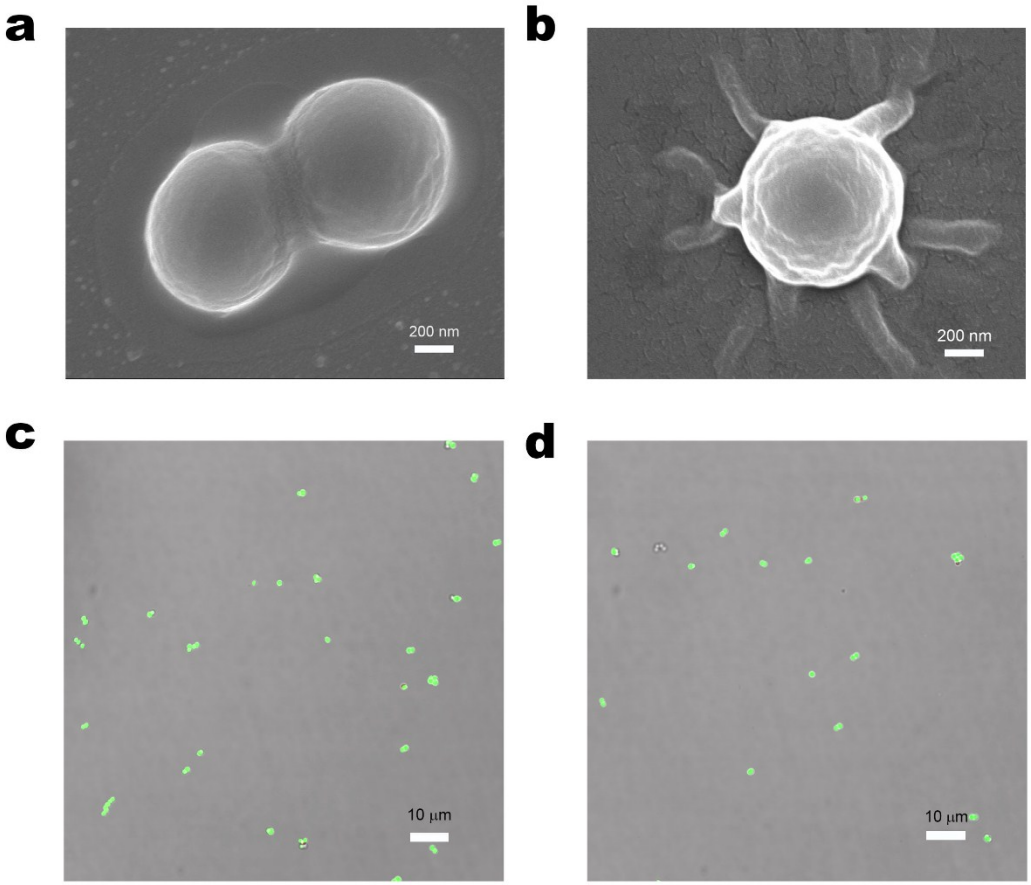

Fig. 12 SEM images of (a) native and (b) coated aureus cell. CLSM images of (a) native and (b) coated aureus cell, which were dyed by SYTO 9. From the results, the surface of coated cells became coarse comparing with native cells, demonstrated the successful coating on *Staphylococcus aureus* cells. Besides, *Staphylococcus aureus* cells also showed good viabilities after coating.