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Polymer–inorganic microcapsules fabricated by combining biomimetic adhesion and bioinspired mineralization and their use for catalase immobilization

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ABSTRACT

Herein, a novel enzyme bioreactor based upon polymer–inorganic hybrid microcapsules was prepared through combining biomimetic adhesion and bioinspired mineralization. Briefly, polydopamine (PDA) as the adhesive layer was formed on the surface of $CaCO_3$ particles through self-polymerization of dopamine. Then, polyethyleneimine (PEI) was anchored on the adhesive layer through Michael addition reaction or Schiff base reaction. Subsequently, titania (Ti) as the mineral layer was generated and deposited on the surface of PDA/PEI-coated particles through the bioinspired mineralization process induced by the free amine groups of PEI. Finally, PDA/PEI/Ti hybrid microcapsules were obtained after removing the $CaCO_3$ templates *via* EDTA treatment. Notably, during the preparation process, PEI could be considered as a "bridge" molecule to connect the adhesive layer and the mineral layer. The chemical/topological structure of the hybrid microcapsules could be facilely tuned by manipulating the molecular weight of PEI. Once utilized for constructing an enzyme bioreactor, the structural evolution of the hybrid microcapsules caused a varied enzyme catalytic activity (42–73 U mg^{-1}). This structure–performance relationship might be due to the alterable diffusion resistance resulting from the mineral layer. Besides, the enzymes encapsulated in PDA/PEI/Ti hybrid microcapsules also exhibited desirable thermal, pH, storage and recycling stabilities.

1. Introduction

During the past decades, biocatalysis, especially enzyme catalysis, has evolved as a promising protocol for the fabrication of commodity chemicals and pharmaceutical intermediates mainly owing to its green and sustainable merits [1,2]. The main shortcomings for industrial application of enzymes are the operation instability and recycling difficulty, which can be overcome through enzyme immobilization [3]. Among numerous immobilization carriers [4–9], microcapsules with a semi-permeable wall and a lumen have gained great attention and already been utilized as an enzyme bioreactor. Theoretically, the capsule lumen could be loaded with a large quantity of enzymes, whereas the semi-permeable capsule wall could allow the diffusion of substrate(s) and product(s) [6]. In general, ideal microcapsules for constructing an enzyme bioreactor should possess good biocompatibility, moderate hydrophilicity, suitable wall structures, and robust mechanical stability. Moreover, the microcapsules should be prepared under mild conditions to ensure the high catalytic activity and stability of the encapsulated enzymes.

Recently, bioinspired/biomimetic approaches (such as bioinspired mineralization [10], biomimetic adhesion [11], and self-assembly [12,13].), which were commonly conducted under mild conditions, have been widely exploited to synthesize hierarchical and multifunctional materials [14–17], including flowers, capsules, tubes, etc. Among, bioinspired mineralization, a process mimic of biomineralization by which soft living organisms create hierarchical hybrid materials [18], has been extensively investigated during the past two decades and already utilized for the preparation of microcapsules. Till now, inorganic oxides [19–21] (such as silica, and titania) and calcium salts [22,23] (such as calcium carbonate and calcium phosphate) as two typical minerals, have

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been successfully incorporated into the capsule wall through bioinspired mineralization. Considering the pH-dependent solubility of calcium salts, microcapsules composed of these calcium salts would be dissolved under acidic conditions. Fortunately, most inorganic oxides-based microcapsules possess high structural stability under either acidic or basic conditions, which could be more suitable for constructing an enzyme bioreactor. For example, Chu et al. [24,25] implemented a bioinspired silicification process on the surface of alginate microcapsules, then acquiring the alginate/protamine/silica hybrid microcapsules. The as-formed silica layer could effectively reinforce the mechanical stability and inhibit the swelling of the capsules. When utilized as the enzyme bioreactor, enhanced recycling and storage stability were obtained. However, the longer diffusion distance of substrate(s) from the bulk solution to the enzyme may cause higher diffusion resistance, then lowering the apparent catalytic activity. To address this issue, $CaCO_3$ particles with a size of several micrometers were adopted as the sacrificial templates to synthesize protamine/titania microcapsules through the synergy between bioinspired mineralization and layer-by-layer assembly [20]. Yeast alcohol dehydrogenase (YADH) was *in situ* encapsulated into the protamine/titania microcapsules, and desirable catalytic activity was acquired. However, the mechanical stability of the resultant microcapsules was very sensitive to the layer number. Besides, the increased layer number would lead to enhanced mechanical stability. Nevertheless, such process was generally labor-intensive and complicated, and the diffusion resistance would simultaneously become larger with the increase of the layer number. To solve these problems, biomimetic adhesion was introduced and integrated with bioinspired mineralization to prepare the hybrid microcapsules with high mechanical stability [26,27]. Specifically, Zhang et al. [27] firstly immobilized cysteamine on polydopamine (PDA)-coated $CaCO_3$ particles. Then, bioinspired titanification was induced by the amine groups of cysteamine. After removal of the $CaCO_3$ templates, polydopamine/cysteamine/titania (PDA/cysteamine/Ti) hybrid microcapsules with enhanced mechanical stability were acquired. The preparation process was simplified and much easier to control in comparison to the method of the synergy between bioinspired mineralization and layer-by-layer assembly. Moreover, the incorporation of PDA expanded the range of templates with different surface properties [28]. Nevertheless, the as-prepared enzyme bioreactor possessed a rather dense capsule wall, which showed high diffusion resistance of substrates, thus leading to low catalytic activity of the encapsulated enzymes. Therefore, it is necessary to seek for effective methods to manipulate the structure of the capsule wall, which may further expand the applicability of this combined biomimetic/bioinspired approach.

In this study, polydopamine/polyethyleneimine/titania (PDA/PEI/Ti) hybrid microcapsules were constructed as an enzyme bioreactor through the combination of biomimetic adhesion and bioinspired mineralization. Firstly, an adhesive layer (PDA) was formed on the surface of $CaCO_3$ particles through self-polymerization of dopamine. Then, PEI was anchored onto the adhesive layer to subsequently induce the bioinspired mineralization for forming the mineral layer (titania). Finally, PDA/PEI/Ti hybrid microcapsules were obtained after removing the $CaCO_3$ templates through EDTA treatment. Notably, PEI molecules acted as a bridge between the adhesive layer and the mineral layer, which could be utilized for manipulating the structure of the microcapsules. Once adopted for immobilizing catalase (CAT), the relationship between capsule structure and catalytic performance was elucidated by measuring the specific activity of CAT encapsulated in different hybrid microcapsules. Besides, the kinetic parameters as well as the stabilities for CAT immobilized in the structure-optimized microcapsules were also investigated.

2. Materials and methods

2.1. Materials

Fluorescein isothiocyanate (FITC), catalase from bovine liver (CAT, 2–5 kU mg^{-1}, EC.1.11.1.6), sodium polystyrene sulfonate (PSS, M_w *ca.* 70,000 Da), titanium(IV) bis-(ammonium lactate)dihydroxide aqueous solution (50 wt%, Ti-BALDH) and polyethyleneimine (PEI; M_w *ca.* 600, 1800, 3500, 70,000 Da) were obtained from Sigma–Aldrich Chemical Corporation. Calcium chloride ($CaCl_2$), ethylenediaminetetraacetic acid (EDTA), sodium carbonate (Na_2CO_3), hydrogen peroxide (H_2O_2, 30 wt%) and tris(hydroxymethyl) aminomethane (Tris) were purchased from Guangfu (Tianjin, China). Dopamine was provided by China Wuhan Yuancheng Technology Development Company Limited. High purity deionized water was obtained from the Millipore Milli-Q water purification system. The pH values of the buffers were adjusted with hydrochloric acid (100 mM) and sodium hydroxide (100 mM).

2.2. Preparation of PDA/PEI/Ti hybrid microcapsules

PSS-doped $CaCO_3$ particles were utilized as the templates, which were prepared according to previous literature [29]. Briefly, 10 mL of Na_2CO_3 (330 mM) solution was poured into 10 mL of PSS-containing $CaCl_2$ (330 mM) solution under vigorous stirring for 25 s. After standing for 10 min, the particles was gathered by centrifugation, and washed twice with water. Then, PSS-doped $CaCO_3$ particles (4–6 μm in diameter) were obtained.

Subsequently, the $CaCO_3$ particles were dispersed in 20 mL of Tris–HCl buffer (100 mM, pH 8.5) with 2 mg mL^{-1} dopamine hydrochloride. The suspension was incubated at ambient temperature for 8 h under constant stirring. After collected through centrifugation and washed twice with water, the PDA-coated $CaCO_3$ particles were obtained. Next, the PDA-coated $CaCO_3$ particles were re-dispersed in 5 mg mL^{-1} PEI aqueous solution with an average molecular weight of 600, 1800, 3500 or 70,000 Da and incubated for 15 min. After centrifugation and washing, particles coated with PEI-grafted PDA layer were acquired. The obtained particles were dispersed into 40 mL of Ti-BALDH aqueous solution (1.25 wt%) and kept under constant stirring for 2 h. Then, to remove the $CaCO_3$ templates, the particles were collected and dispersed in 30 mL, 50 mM EDTA aqueous solution and gently stirred for 10 min. PDA/PEI/Ti hybrid microcapsules were finally obtained after centrifugation and washing with water. Besides, the pristine PDA microcapsule was also prepared through the procedure as mentioned above in the absence of PEI and Ti-BALDH.

2.3. Characterizations

Scanning electron microscope (SEM, Nanosem 430) was conducted to characterize the morphologies of PDA/PEI/Ti and PDA microcapsules. Energy dispersive spectroscope (EDS) attached to SEM was utilized to analyze the elements of PDA/PEI/Ti and PDA microcapsules. The Fourier transform infrared spectroscopy (FTIR) spectrum was obtained by using the Nicolet-6700 spectrometer. Thermogravimetric (TGA) experiments were conducted by using Perkin-Elmer Pyris analyzer. The location and distribution of CAT in the capsule lumen were recorded by using Confocal Microscope (LSM 710). The catalytic activity and immobilization efficiency of enzyme were determined by using UV–vis spectrophotometer (Hitachi U-3010).