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Mussel-Inspired Encapsulation and Functionalization of Individual Yeast Cells

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Supporting Information

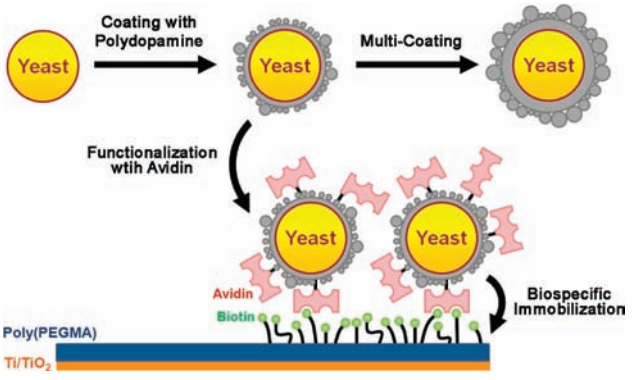
ABSTRACT: The individual encapsulation of living cells has a great impact on the area of cell-based sensors and devices as well as fundamental studies in cell biology. In this work, living yeast cells were individually encapsulated with functionalizable, artificial polydopamine shells, inspired by an adhesive protein in mussels. Yeast cells maintained their viability within polydopamine, and the cell cycle was controlled by the thickness of the shells. In addition, the artificial shells aided the cell in offering much stronger resistance against foreign aggression, such as lyticase. After formation of the polydopamine shells, the shells were functionalized with streptavidin by utilizing the chemical reactivity of polydopamine, and the functionalized cells were biospecifically immobilized onto the defined surfaces. Our work suggests a biomimetic approach to the encapsulation and functionalization of individual living cells with covalently bonded, artificial shells.

In nature, certain biological systems, including bacteria, plants, algae, and fungi, have evolved to preserve their species under unfavorable harsh environments by protecting their genetic information with a hard shell. Besides the biological uniqueness, the robustness of such shells was believed to be beneficial in increasing the stability of cell-based devices, including sensors: for example, microbial spores have been used for analyte detection[1] and cell patterns.[2] On the other hand, sporelike structures have recently been generated chemically by encapsulating individual living cells within artificial shells, such as silica,[3] calcium phosphate,[4] calcium carbonate,[5] and multilayers of polyelectrolytes.[6] We anticipate that these artificially formed sporelike structures ("artificial spores") will contribute to increasing the long-term stability and performance of cell-based sensors, bioreactors, microfluidic devices, etc., as well as to fundamental studies in cell biology. Most of the methods for forming artificial shells have been limited to electrostatic layer-by-layer (LbL) self-assembly of polyelectrolytes, where polyelectrolyte multilayers acted as a shell material itself[6] or a catalytic template for subsequent formation of inorganic shells.[3,4]

Cell-surface modification is another important issue for the application of living cells. It has been achieved mostly by complicated methods, such as the introduction of nonbiogenic functional groups by metabolic or genetic engineering.[7] Noncovalent adsorption of macromolecules onto the cell surfaces also has been attempted in order to introduce chemical functionalities into living cells.[7e,8] However, cell-surface modification has not been achieved simultaneously with protective encapsulation. In this work, we report a simple but versatile approach—inspired by nature—for encapsulating individual living cells with functionalizable, artificial organic shells formed by strong covalent bonds. Polydopamine (PD), inspired by an adhesive protein in mussels,[9] was chosen as a coating material for introducing organic shells onto living cells (Scheme 1). The polymerization of dopamine occurs under biologically compatible conditions, and PD itself exhibits negligible cytotoxicity, as demonstrated by the fact that it promotes cell adhesion on various substrates.[10,11] In addition, we envisioned that the chemical reactivity of the PD shells formed on individual living cells could be utilized for cell-surface postmodification.[9b−9d]

Dopamine was polymerized on the surface of *Saccharomyces cerevisiae* (yeast),[9,10] leading to the formation of single PD-coated yeast cells (yeast@PD_1). The same procedure was repeated with yeast@PD_1 to form double-PD-coated yeasts (yeast@PD_2). The yeast cells were encapsulated separately and individually within PD shells (Figure 1a,b; also see the Supporting Information for

Scheme 1. Schematic Representation of Polydopamine Encapsulation of Individual Yeast Cells and Functionalization of the Artificial Shells



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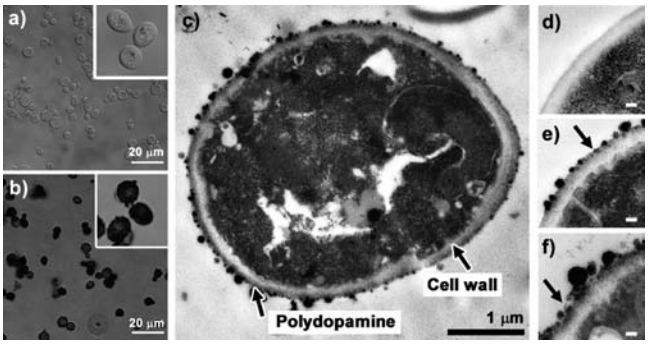

Figure 1. Confocal micrographs of (a) native yeasts and (b) yeast@PD_2. The insets show magnified images. (c) TEM micrograph of microtome-sliced yeast@PD_1. (d–f) Magnified TEM images of (d) native yeast, (e) yeast@PD_1, and (f) yeast@PD_2 (scale bar = 100 nm). The arrows indicate the polydopamine shells.

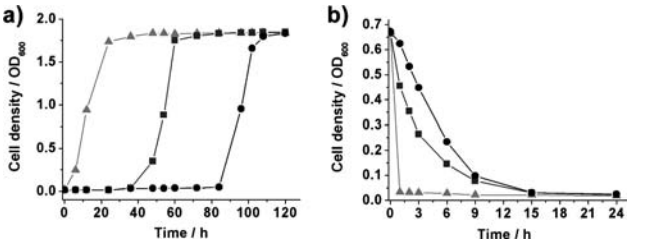

Figure 2. (a) Growth curve of native yeast (▲), yeast@PD_1 (■), and yeast@PD_2 (●). (b) Survival of native yeast (▲), yeast@PD_1 (■), and yeast@PD_2 (●) in the presence of lyticase. Optical density was measured using absorbance measurements at 600 nm (OD_{600}).

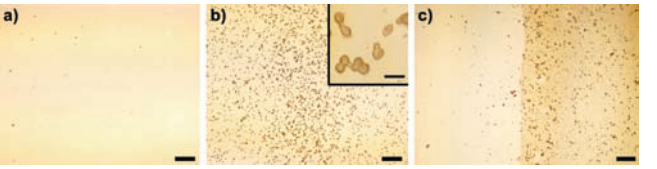

Figure 3. Optical micrographs of (a) yeast@PD_1 and (b) avidin-linked yeast@PD_1 on biotin-presenting poly(PEGMA) surfaces. (c) Optical micrograph of avidin-linked yeast@PD_1 on a biotin-patterned poly(PEGMA) surface that was composed of a poly(PEGMA) region (left) and a biotin-presenting region (right). The scale bars in the main panels represent 100 μm, and that in inset of (b) represents 10 μm.

confocal images of yeast@PD_1). Yeast@PD was opaque to light because of its PD shell. The opacity results from the molecular structure of PD, which is thought to be composed of highly conjugated aromatic rings.[9d] While the native yeast was noticeably shrunk, yeast@PD maintained its original round shape even after drying for 12 h at room temperature (see the Supporting Information for scanning electron microscopy images). The maintenance of the shape indicated that the PD shells could act as a physically protective shell for living cells.[3−5] Transmission electron microscopy (TEM) micrographs showed that the PD shell consisted of two parts, a uniform thin film firmly coating the cell wall and big particulates (Figure 1c−f; also see the Supporting Information for a TEM image of the native yeast). The robust coating was achieved presumably by covalent bonding between PD and amine or thiol moieties of (glyco)proteins in the cell wall during the course of polymerization.[9b−9d,12] In addition, the results showed that the thickness of the PD shell was controlled facilely by multicoating: the average thicknesses were estimated to be ~30 nm for yeast@PD_1 and ~80 nm for yeast@PD_2 These values corresponded well with those for previously reported PD films that had been formed on flat surfaces and microspheres.[9d,10]

The PD encapsulation of yeast cells was found to control cell division, which would be one of the essential characteristics to be drawn from the artificially formed sporelike structures. The cell-culture experiments showed that yeast cells kept the capability of dividing themselves under culture conditions even after PD encapsulation (Figure 2a; also see the Supporting Information for confocal micrographs of duplicating cells). Native yeast cells immediately proliferated without a lag phase, but the growth curves of yeast@PD_1 and yeast@PD_2 remained in the lag phase for more than 24 h. Since the viability was much above 50% (see the Supporting Information for the viability test), we thought that the PD shell prevented yeast cells from dividing and let them remain in a quiescent state, because even 50% loss of the living cells would just extend the lag phase slightly. More interestingly, the period of the quiescent state was controlled by the thickness of the PD shell: the lag phase of yeast@PD_1 lasted for 36 h, while that of yeast@PD_2 lasted for 84 h.

It is one of the important roles of hard shells in natural spores to preserve their species against foreign aggression. Although both native and PD-coated yeasts were lysed as a result of the digestion activity of lyticase, the PD-coated yeasts were much more resistant against lysis than the native yeast: ~70% of yeast@PD_1 and ~90% of yeast@PD_2 still survived after 1 h, while more than 90% of the native yeast was lysed (Figure 2b; also see the Supporting Information for full UV spectra). The results clearly show that the PD shells retarded the penetration of lyticase, probably by stabilizing cellular membranes through the formation of covalent bonds between PD and cell-wall constituents. Furthermore, the resistance against lyticase was controlled simply by the thickness of the PD shell. The optical density of yeast@PD_2 remained higher than that of yeast@PD_1 until 15 h. The control of cell-division cycles or chemical/biological protection was not reported for organic multilayers of polyelectrolytes,[6] implying that the multilayers were too weak to protect the cells and suppress cell division because they were formed by electrostatic interactions. Therefore, the PD encapsulation, which utilizes covalent bonds, could serve as a new strategy for controlling cell division and protection of artificial sporelike structures in a designed way.

We utilized the chemical reactivity of PD toward amine and thiol functionalities[9b−d] for the surface modification of yeast@PD_1.[13] Yeast@PD_1 was functionalized with avidin and then immobilized on a biotin-presenting surface (see the Supporting Information for the detailed experimental procedures with native yeast as a control). Avidin-linked yeast@PD_1 was densely immobilized onto a biotin-presenting poly(PEGMA) surface, while yeast@PD_1 was rarely adsorbed onto the same surface (Figure 3a,b). When avidin-linked yeast@PD_1 was exposed to the biotin-patterned poly(PEGMA) surface, it was conjugated spatioselectively only onto the biotin-presenting area via the biospecific interaction between avidin and biotin (Figure 3c).

In summary, we have demonstrated the reactive encapsulation of individual yeast cells with polydopamine, which is a biocompatible coating material inspired by the adhesive protein in mussels. The individual encapsulation with polydopamine is of importance in the realization of artificial spores: (1) It is the first approach to the encapsulation of living cells within covalently bonded organic materials. (2) The polydopamine coating was found to be physically stable in comparison with polyelectrolyte multilayers and to be

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effective in protecting living cells and controlling cell division. (3) The polydopamine shell could be further functionalized for applications of interest. In comparison with previous reports,[3−7] polydopamine encapsulation is a simple and versatile method for introducing various functionalities onto the cell surface under physiologically compatible conditions. We believe that polydopamine encapsulation would be a good starting point for both fundamental research and applications based on artificial spores, as it endows living cells with durability against harsh environments, controllability in cell cycles, and reactivity for cell-surface modification.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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