Biomimicry

DOI: 10.1002/ange.201102030

Bioinspired Functionalization of Silica-Encapsulated Yeast Cells**

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Dedicated to Professor Eun Lee on the occasion of his 65th birthday

Cell-surface modification is usually achieved by sophisticated but complicated methods, such as the introduction of non-biogenic functional groups by metabolic or genetic engineering.[1] Although such methods have evolved into biocompatible and bioorthogonal strategies, the possibility that the direct insertion of functional moieties causes significant perturbations to cell membranes still remains. For a decade, encapsulation methods have been developed as an alternative, indirect approach to cell-surface modifications, as it is thought that the cell integrity would not be perturbed by the encapsulation methods where functional moieties are introduced onto the cell surface without any direct contact with cell membranes. For example, the noncovalent adsorption of macromolecules,[2] mostly by layer-by-layer (LbL) processes,[3] has been utilized to introduce various functionalities, including fluorescent and magnetic properties, catalytic moieties, and supporting templates, to the living cells. On the other hand, recently reported artificial shells, which robustly encapsulate individual living cells,[4–7] have attracted a great deal of attention as a new approach to cell-surface modifications and formation of artificial spores, because the artificial shells were reported to enhance cell viability and also to control cell division;[4–6] these factors would be beneficial in the development of biosensor circuits, lab-on-a-chip systems, and bioreactors, as well as for fundamental studies in cell biology. It is therefore anticipated that the synergistic combination of the protective encapsulation and the cell-surface functionalization would make a significant step towards the aforementioned applications.

Despite the advantages of physically protective shells, the utilization of the artificial shells for practical applications still remains a challenge. The mechanical robustness and chemical inertness of the artificial shells prove beneficial for protecting living cells, but, contradictorily, these properties limit chemical functionalizations of the shells in terms of reactivity. For example, calcium carbonate or calcium phosphate shells lack chemical reactivity. Although the chemistry of silicon is well established, the functionalization of silica shells requires harsh conditions, such as high pH values and harmful solvents. Therefore, it is a prerequisite for any application that the functionalizabilty of the artificial shells is ensured along with the mechanical robustness of the protective shells. Herein we report a bioinspired method for the encapsulation of individual living yeast cells with functionalizable silica shells. Specifically, we used biomimetic silicification,[8] which was inspired by the biosilicification of diatoms.[9]

Biomimetic silicification is achieved by specific interactions between silicic acid derivatives and cationic polyamines, such as natural and synthetic peptides, and synthetic polymers: the self-assembled structure of polyamines is thought to act as a catalytic template for the in vivo polycondensation of silicic acid derivatives.[8–10] We reasoned that chemical functional groups would be introduced directly to the biomimetically formed silica by adding silanol derivatives that contain functional groups in the course of biomimetic polycondensation of silicic acid derivatives. (3-Mercaptopropyl)trimethoxysilane (MPTMS) was selected as a model additive because it was reported to be polycondensed simultaneously with silicic acid under physiologically mild conditions.[11,12] The functionalizable silica shells formed in this work would expand the utility of artificial shells, because the thiol group in the silica shell can be used for introducing various functions through specific reactions of the thiol moiety with maleimide derivatives under biocompatible conditions (aqueous solution, pH 7.4; Figure 1).[13]

The polyelectrolyte multilayer of poly(ethyleneimine) (PEI, M_w: 750000) and poly(sodium 4-styrenesulfonate) (PSS, M_w: 70000) was used as a catalytic template for biomimetic silicification because previous studies indicated that PEI was biocompatible[14] and acts as a catalyst for biomimetic silica formation.[11,15] PEI and PSS were alternately deposited onto the surface of *Saccharomyces cerevisiae* (*S. cerevisiae*; baker's yeast). The layer-by-layer processes were initiated with PEI so that electrostatic interactions occur with the negatively charged cell surfaces, and terminated with PEI so that catalytic interactions occur with silicic acid derivatives at the outer interface. For the individual encapsulation of yeast cells with thiol-functionalized silica (SiO_2^{SH}; i.e., formation of yeast@SiO_2^{SH}), the PEI/PSS multilayer-coated cells were placed for 30 min in a silicic acid derivative solution (100 mM), which had been prepared by adding

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[**] This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, KRF-2008-313-C00496) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0001953). We thank M. S. Hyun and M. H. Kim at the National Nanofab Center for the SEM analyses, K.-B. Lee at KBSI for the TEM analyses, and S. M. Kang at KAIST for poly(PEGMA) synthesis.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201102030.

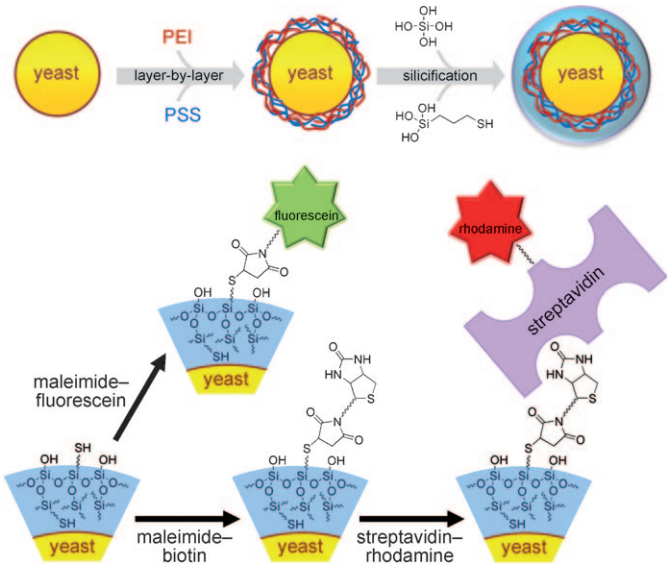

Figure 1. Encapsulation of individual yeast cells in the thiol-functionalized silica (SiO_2^{SH}) shells and the introduction of fluorescein or streptavidin onto the SiO_2^{SH} shells.

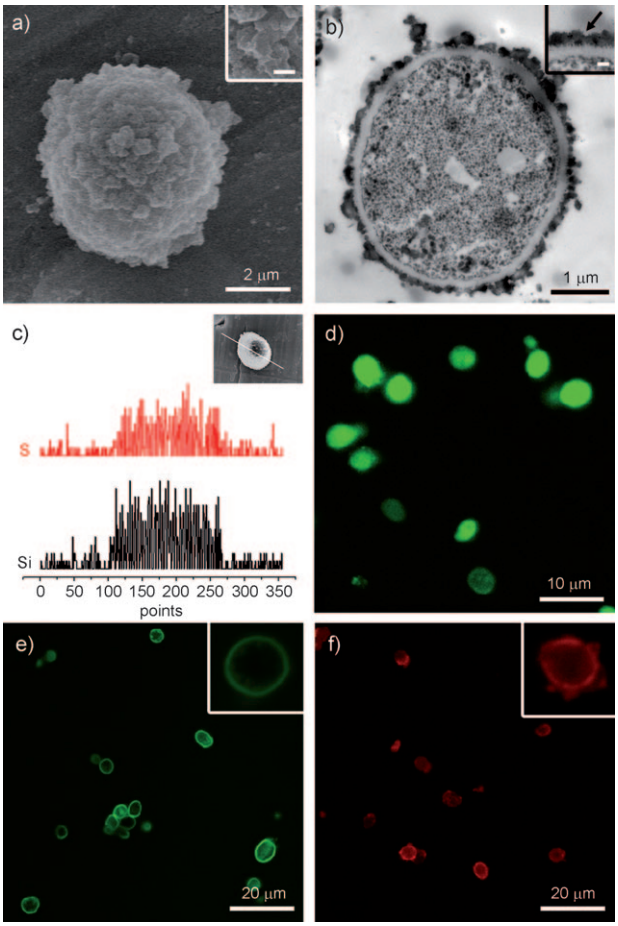

Figure 2. a) SEM micrograph of yeast@SiO_2^{SH} after 12 h drying at room temperature. b) TEM micrograph of microtome-sliced yeast@SiO_2^{SH}. Insets in (a) and (b) show magnified images of the SiO_2^{SH} shells (arrow indicates the SiO_2^{SH} shell; scale bar: 100 nm) c) EDX line profiles of yeast@SiO_2^{SH} for silicon and sulfur. d) Confocal microscopy image of yeast@SiO_2^{SH} stained by fluorescein diacetate for viability testing. Confocal microscopy images of e) fluorescein-functionalized and f) rhodamine-linked streptavidin-functionalized yeast@SiO_2^{SH}. Insets in (e) and (f) show magnified images.

hydrolyzed solutions of tetramethyl orthosilicate (TMOS; 1M) and MPTMS (1M) to phosphate buffer (50 mM, pH 7.4) in a 25:75:900 (v/v/v) ratio.[11,12]

Field-emission scanning electron microscopy (FE-SEM) confirmed that the yeast cells were encapsulated separately and individually within the SiO_2^{SH} shells, and maintained their structural integrity after encapsulation (Figure 2a,b; see the Supporting Information for SEM images of native yeasts, multilayer-coated yeasts, and yeast@SiO_2^{SH}, and for TEM images of native yeasts). The high-magnification SEM micrograph (Figure 2a, inset) showed that the surface was composed of silica nanoparticles that had been observed in previous studies of biomimetic silicification.[5,8] The transmission electron microscopy (TEM) micrograph of microtome-sliced yeast@SiO_2^{SH} indicated that cytoplasm maintained its inherent integrity, and the artificial shell intimately covered the cell wall (Figure 2b). The average thickness of the SiO_2^{SH} shell was estimated to be approximately 140 nm (Figure 2b, inset). The successful incorporation of MPTMS and the presence of the thiol group were confirmed by the line-scan analysis of energy-dispersive X-ray (EDX) spectroscopy (Figure 2c; see the Supporting Information for EDX analysis of PEI/PSS multilayer-coated yeasts and for detailed elemental analysis data).

Biocompatibility of artificial materials or modification protocols is a crucial factor for the use of the materials or protocols for biological systems. Hence we tested the viability of the encapsulated cells by using fluorescein diacetate, which is used to examine the activity of intracellular esterases and membrane integrity (Figure 2d; see the Supporting Information for the viability test of native yeasts and for brightfield images).[3a,4,7] Green fluorescence in the cytoplasm indicated that the cells maintained their viability after the LbL and silicification processes. In addition, the viability test indicated that 1) the encapsulation processes were compatible with living yeast cells and 2) the artificial shell would be permeable to small molecules,[4–7] such as water or nutrients, as inferred from the permeability of fluorescein diacetate.

The functionalizable SiO_2^{SH} shells would allow the introduction of various functions to biological systems, with the successful formation of the shells without any significant disturbance of the viability of living cells. As a demonstration, the fluorescent dye fluorescein was introduced to the SiO_2^{SH} shell by using a fluorescein-linked maleimide (Figure 2e; see the Supporting Information for brightfield images of native yeasts as a control). The ring-shaped green fluorescence clearly indicated that the cell was functionalized with fluorescein (Figure 2e, inset). The results confirmed the functionalizability of the thiol group in the SiO_2^{SH} shell. In addition, we chemospecifically immobilized streptavidin onto yeast@SiO_2^{SH} by sequential specific reactions (Figure 2f; see the Supporting Information for brightfield images of native yeasts as a control). The biotinyl functional group was introduced by coupling the thiol group with a biotin-linked

maleimide, and then rhodamine-linked streptavidin was conjugated onto the biotin-functionalized yeast@SiO_2^{SH}. The ring-shaped red fluorescence confirmed that the yeasts were functionalized with streptavidin (Figure 2 f, inset). The results showed that the thiol functional group in the SiO_2^{SH} shell could be used for introducing various functions, such as fluorescent dyes, chemical moieties, or proteins, onto the silica surfaces by utilizing the specific reaction between thiol and maleimide derivatives in aqueous solution at pH 7.4.

Furthermore, the site-specific immobilization of living cells onto the surface is an important step for the fabrication of cell-based sensors. As such, the biotin-functionalized yeast@SiO_2^{SH} was further utilized for immobilization onto defined surfaces functionalized with poly(polyethyleneglycol methacrylate) (poly(PEGMA)), which has non-biofouling properties (see the Supporting Information for the detailed synthetic scheme and procedures).[16] The biotin-functionalized yeast@SiO_2^{SH} could be densely immobilized onto an avidin-functionalized poly(PEGMA) surface, while it was hardly adsorbed onto a biotin-functionalized poly(PEGMA) surface (Figure 3 b, c; see the Supporting Information for

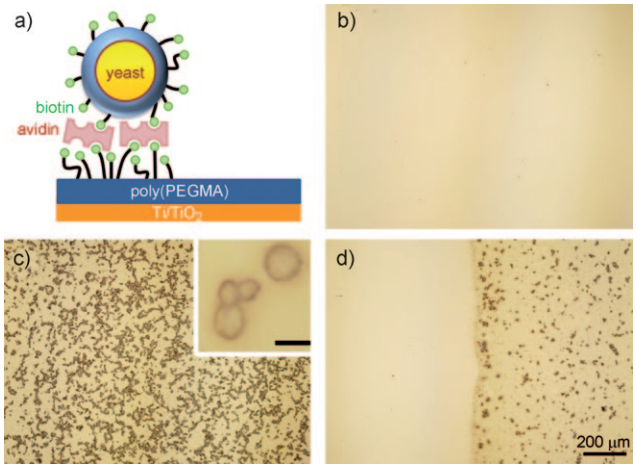

Figure 3. a) Schematic representation of the bioselective attachment of yeast@SiO_2^{SH} onto the surface. Optical micrographs of b) biotin-functionalized yeast@SiO_2^{SH} on the biotin-functionalized poly(PEGMA) surface, c) biotin-functionalized yeast@SiO_2^{SH} on the avidin-functionalized poly(PEGMA) surface (inset: magnified image; scale bar: 10 μm), and d) biotin-functionalized yeast@SiO_2^{SH} on the avidin-patterned poly(PEGMA) surface that is composed of the avidin-functionalized region (left) and poly(PEGMA) region (right).

native yeast as a control). The high-magnification image showed that each cell was firmly attached to the surface (Figure 3 c, inset). When biotin-functionalized yeast@SiO_2^{SH} was exposed to the avidin-patterned poly(PEGMA) surface, it was conjugated spatioselectively only onto the biotin-functionalized area through the biospecific interaction between avidin and biotin (Figure 3 d).

In summary, we have reported a biomimetic approach to the functionalization of the silica-encapsulated yeast cells under physiologically mild, biocompatible conditions, inspired by the biosilicification of diatoms. The silica encapsulation and thiol functionalization were simultaneously achieved, and chemical/biological functionalities were subsequently introduced by the maleimide-based coupling. The resulting "artificial spores" could find their own applications in various areas, such as cell-based sensors, reactors, and devices, as well as single-cell-based biology. We believe that our work can greatly contribute to this emerging field, and is a first step towards the controlled manipulation of individual cells.

Received: March 22, 2011
Published online: May 30, 2011

Keywords: biomimetic synthesis · encapsulation · layer-by-layer processes · silica · yeast cells

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