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COMMUNICATION

Peptide-Catalyzed, Bioinspired Silicification for Single-Cell Encapsulation in the Imidazole-Buffered System

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Inspired by biosilicification of glass sponges, we designed a catalytic peptide, which formed silica structures in the imidazole-buffered solution. The peptide was adsorbed selectively onto the surface of yeast cells, and the bioinspired silicification led to the formation of a cytoprotective silica shell on individual yeast cells.

Certain organisms, such as diatoms¹ and glass sponges,² have evolved to protect their cellular entities with siliceous exoskeletons. A phylum ciliophora *Maryna umbrellata* is another example of the organisms that construct silica structures in nature: it adapts itself to external stresses, such as desiccation and starvation, and forms an outmost silica-particulate layer at the resting cyst state.³ Researchers have been inspired to chemically mimic these biological processes and to generate hierarchical and structurally-intricate silica structures under physiologically mild conditions by using a synthetic catalyst the molecular structure and function of which mimic the natural counterparts.⁴ The cytocompatible conditions for bioinspired silicification are greatly beneficial in dealing with living cells, and have recently been applied to the formation of a cytoprotective, ultrathin (< 100 nm) silica coat onto individual microbial⁵ and mammalian cells⁶ with an aim of endowing the cells with enhanced resistance against external stressors as well as manipulating the cellular activities at the single-cell level. Especially, the diatom-inspired silicification has intensively been applied to single-cell silica encapsulation by utilizing the polyamines, such as poly(diallyldimethylamine) or poly(ethyleneimine), as a catalytic template.⁷ In a similar process, the artificial shells of abiological titania⁸ and silica-titania⁹ were also formed on individual living cells by using the arginine (Arg) and lysine (Lys)-rich peptide that had high affinity to titanium oxide. However, these approaches generally involved time-consuming, multi-step layer-by-layer processes for introduction of the catalytic template onto cell surfaces.¹⁰

It was reported that biosilicification in glass sponges was catalysed by silica-forming enzymes (silicateins), and the biochemical studies revealed that their catalytic activity required histidine (a general base) and serine (a nucleophile) at a proximate position in the active site, reminiscent of human protease cathepsin L.¹¹ Accordingly, biomimetic analogues were proposed to emulate biosilicification activities of the silicateins, such as diblock copolypeptides¹² and bifunctional small molecules.¹³ The in vitro

silicification studies found that the cysteine-lysine-containing copolypeptide and cysteamine were catalytically active for silica formation at near neutral pH. In this work, we introduced minimal motifs mimicking silicateins and cell-anchoring ones to a peptide sequence for the glass sponge-inspired silica-shell formation by a one-step adsorption of the catalytic peptide onto a cell surface. This simple adsorption of the peptide could allow for circumventing cell aggregation and/or cell death resulting from the repeated interactions with cytotoxic polyamines.¹⁴ In addition, the collection yield of encapsulated cells would be increased by the simplified encapsulation process.

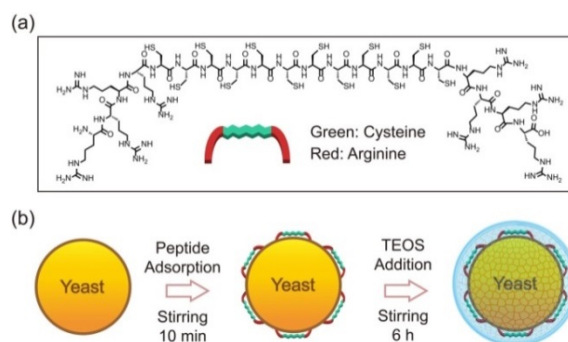


Fig. 1 (a) Molecular structure of R₄C₁₂R₄. (b) Schematic representation for silica encapsulation of individual cells.

We rationally designed the peptide sequence, R₄C₁₂R₄ (R: arginine; C: cysteine), where the basic R₄ sequence was attached to both ends of the catalytic C₁₂ sequence; the positively charged R sequence ($pK_a = 12.0$) ensured the electrostatic adsorption of the peptide onto the negatively charged cell surface. Thiol group in cysteine, mimicking the serine residue in the active site of silicatein- α , was reported to play an important role in initiating the hydrolysis of TEOS, which is known as the rate-limiting step for silicification (Fig. 1).¹³ In addition, we envisioned that imidazole could be used for not only buffering the solution pH but also acting as a counterpart of the catalytic action based on the chemical mimicry of the histidine residue in the active site of silicatein- α . As a first step, the catalytic activity of the peptide in solution was investigated by

reacting tetraethyl orthosilicate (TEOS, 10 mM) and $R_4C_{12}R_4$ (0.1 mg) for 6 h in the imidazole-buffered solution (1 mL, pH 7.4). The white precipitate was observed with naked eyes, and the silicomolybdc acid assay indicated that the amount of polymerized Si was 33.6 $\mu\text{g}/\text{mL}$ based on the interpolation with a linear standard curve (Fig. 2a; Fig. S1 for a linear standard curve, ESI†). The reaction without $R_4C_{12}R_4$ in the imidazole-buffered solution did not generate silica, confirming the catalytic role of $R_4C_{12}R_4$ for the silicification. In contrast to the imidazole-buffered solution, neither Tris- nor phosphate-buffered solution showed a distinct peak at the wavelength of 810 nm, which is the characteristic absorption of the reduced molybdenum blue complex.¹⁵ Although arginine-rich peptide have been used for biomimetic silicification with silicic acid, $R_4C_{12}R_4$ required imidazole buffer to promote the synthesis of silica. We supposed that $R_4C_{12}R_4$ itself might not have enough catalytic activity for silicification with 10 mM of TEOS. The quantitative analysis of silica production implied that imidazole in the buffer might act as an external catalytic pair (general base) of the $R_4C_{12}R_4$ peptide in the silicification processes.¹⁶ The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images indicated that the silica structures were composed of nanometer-sized aggregates (Fig. 2b).

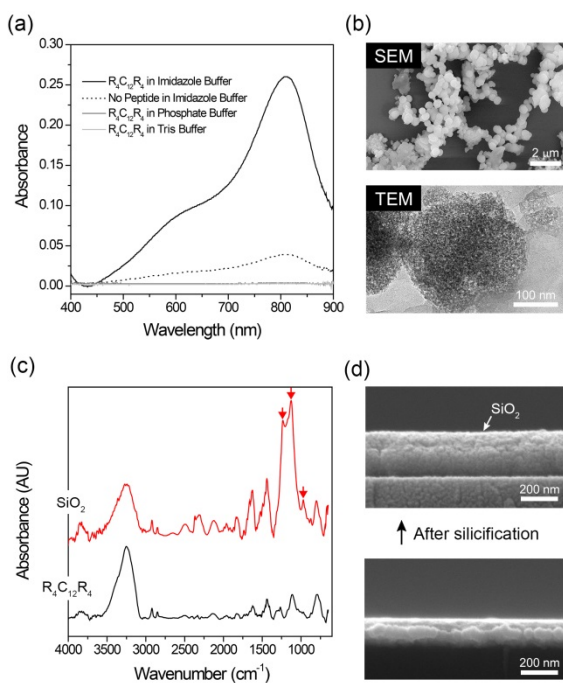


Fig. 2 (a) UV-Vis absorption spectra of molybdenum blue complexes (absorption peak: 810 nm). (b) SEM and TEM micrographs of silica formed by silicification with $R_4C_{12}R_4$ in the imidazole-buffered solution. (c) FT-IR spectra of $R_4C_{12}R_4$ -coated gold substrates (black) before and (red) after silicification. Red arrows indicate the characteristic peaks of silica. (d) SEM cross-section micrographs of (bottom) $R_4C_{12}R_4$ -coated gold (bottom) before and (top) after silicification.

The silicification was also performed with carboxylic acid-terminated self-assembled monolayers (SAMs) on gold after the adsorption of $R_4C_{12}R_4$ to confirm that the one-step adsorption of the peptide was sufficient for the generation of uniform silica films. The Fourier-transform infrared (FT-IR) spectrum showed the characteristic silica peaks at 1228 (Si-O-Si asymmetric stretching), 967 (Si-O stretching), and 803 cm^{-1} (Si-O-Si symmetric stretching) (Fig. 2c).¹⁷ The SEM image indicated that the film thickness was about 80 nm (Fig. 2d). Taken all together, the results confirmed that

the nanometer-thick silica films were formed with the $R_4C_{12}R_4$ peptide under mild conditions in the imidazole-buffered solution.

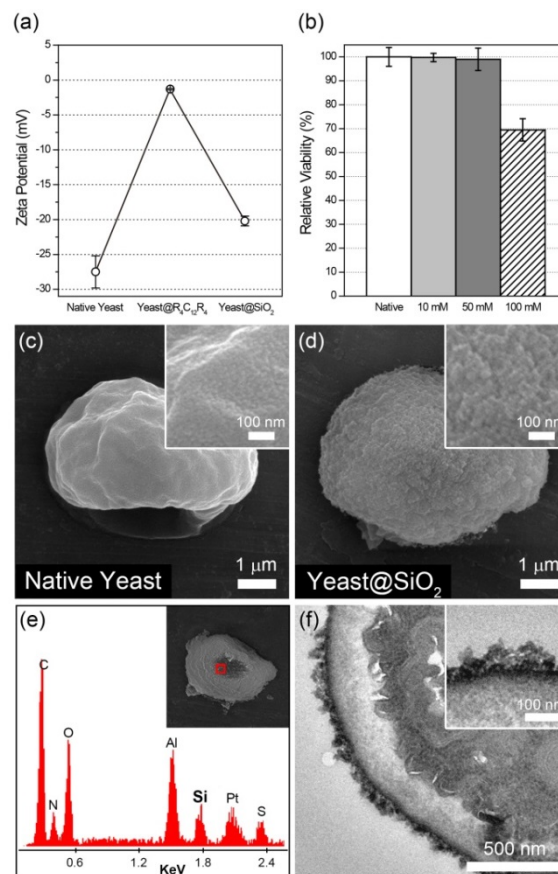


Fig. 3 (a) Zeta potentials of yeast cells after each step. (b) Relative viability of yeast cells after silicification with different concentrations of TEOS. (c,d) SEM micrographs of native yeast and yeast@ SiO_2 . Insets: magnified images. (e) EDX profile of yeast@ SiO_2 for the red square area of the SEM image. (f) TEM micrograph of microtome-sliced yeast@ SiO_2 . Inset: magnified image.

We used *Saccharomyces cerevisiae* (Baker's yeast) for cell encapsulation because it is the simplest eukaryotic organism, but full of possibilities that wild-type strain and mutants could be harnessed in numerous applications, such as single-cell based sensors, microscreening devices, and bioreactors.¹⁸ Yeast cells have the negatively charged outermost membrane consisting of mannoproteins.¹⁹ Therefore, $R_4C_{12}R_4$ was adsorbed onto yeast cell surface via electrostatic interactions by the simple addition of an aqueous $R_4C_{12}R_4$ solution (1 mg/mL) to yeast cells with stirring for 10 min. The adsorption of $R_4C_{12}R_4$ was confirmed by the change in the zeta potential from -27.5 ± 2.3 mV (native yeast) to -1.3 ± 0.1 mV ($R_4C_{12}R_4$ -adsorbed yeast; denoted as yeast@ $R_4C_{12}R_4$) (Fig. 3a). To encapsulate yeast cells with silica, yeast@ $R_4C_{12}R_4$ was collected and re-dispersed in the imidazole-buffered solution (pH 7.4), followed by the addition of TEOS and 6-h incubation, leading to the formation of yeast@ SiO_2 . The zeta potential of yeast@ SiO_2 was restored negatively to -20.2 ± 0.7 mV, indicating the successful formation of silica. The cell viability was found to depend upon the initial concentration of TEOS. The cells were viable with up to 50 mM of TEOS (in comparison with the previous result, it was found that the viability of yeast@ SiO_2 was enhanced),⁵ while the viability sharply dropped to 69% with 100 mM of TEOS (Fig. 3b). Therefore, we used the 50 mM of TEOS for further characterizations of

yeast@SiO₂. The SEM images showed noticeable morphological changes after silica-shell formation (Fig. 3c,d). The nanometer-sized silica particulates were observed on the surface of yeast@SiO₂, and the presence of Si was confirmed by energy-dispersive X-ray (EDX) spectroscopy (Fig. 3e). The thickness of the silica shell was measured to be ~60 nm based on the TEM image of microtomed yeast@SiO₂, which corresponded well to the result with a gold substrate (Fig. 3f).

The single-cell encapsulation has been used for controlling the cell division and protecting the encapsulated cells against harmful external stressors.²⁰ In this work, the cell growth was monitored in yeast extract-peptone-dextrose (YPD) broth media at 30 °C by measuring the optical density at 600 nm (OD₆₀₀) (Fig. S2, ESI†). The plot of ln(OD₆₀₀) vs. time for exponential growth phase showed that the cell-division timing²¹ was increased to 19.1 h from 11.7 h (for native yeast) by forming the silica shell. The slope of linear fitted curves (μ , specific growth rate) was 0.487 h⁻¹ for native yeast and 0.477 h⁻¹ for yeast@SiO₂, indicating the silica shell retarded the cell division timing presumably because of mechanical durability and/or decreased diffusion of nutrients and gases, but did not do harm to metabolic activities for cell growth. The protection ability of the silica shell was tested with an enzyme complex, lyticase. Native yeast or yeast@SiO₂ was incubated for 30 min in a lyticase solution (~38 μ g/mL, \geq 2,000 units/mg protein). While the OD₆₀₀ value fell down sharply to ~0 due to the cell lysis for the native cell, about 30% of the initial OD₆₀₀ value was still maintained for yeast@SiO₂, indicating the cytoprotective property of the silica shell (Fig. S3, ESI†).

In summary, we designed a short peptide, which was adsorbed electrostatically onto the cell surface and catalyzed the silicification of TEOS on the cell surface in the imidazole-buffered solution. Compared with the multi-step layer-by-layer approaches to the introduction of catalytic templates onto cell surfaces, the method demonstrated herein requires only one step for adsorption, which is beneficial to the efficiency enhancement in cell encapsulation processes. Our peptide-based approach to silica formation would widen the chemical tools for single-cell encapsulation.^{7,22} We, especially, believe that the material scope would be expanded to other biogenic or abiological inorganics besides silica with a proper modification of catalytic peptides.²³ Interfacing living cells with various inorganic materials such as silica, titania, calcium carbonate, and calcium phosphate will draw more attention,²⁴ because the resulting inorganic shells could endow living cells with tailored functions, including mechanical hardness, surface modification, and selective permeability for biomedical applications (e.g., bioreactors, biosensors, and microdevices).²²

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Notes and references

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† Electronic Supplementary Information (ESI) available: Detailed experimental procedures, linear standard curve for silicomolybdic acid assay, linear-fitted plot for determination of division timing, and optical density changes after lyticase treatment. See DOI: 10.1039/c000000x/

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