Cytocompatibility Optimization for Silica Coating of Individual HeLa Cells

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Diatoms, which are unicellular organisms living in fresh and marine waters, are encased within a biogenic siliceous shell, which has superior properties such as physical rigidity and chemical stability.[1] Over the past decade, biosilicification processes of diatoms have attracted biologists and materials scientists, because structurally exquisite silica is formed under physiologically mild conditions.[2] It is reported that the biosilicification is achieved by specific interactions between silicic acid derivatives and silaffins, which are posttranslationally modified peptides containing cationic polyamines.[3] Inspired by the silica formation in diatoms, silica nanostructures including nanoparticles and thin films have been formed *in vitro* under mild conditions, such as near-neutral pH, aqueous solution, ambient pressure, and low concentration of silicic acids, by utilizing catalytic templates composed of synthetic macromolecules.[4]

By taking full advantage of cytocompatibility in biosilicification processes, we encapsulated individual living microbial cells with silica while maintaining the cell viability.[5,6] The microbial encapsulation was achieved by two sequential steps: introduction of a catalytic template, and bioinspired silicification. Microbial cells, such as yeast, *Escherichia coli*, and endospore, were encapsulated chemically within 50-nm-thick silica shells by mimicking the silica-forming process of diatoms, resulting in the enhancement of long-term viability.[5] We have also reported another bioinspired approach to the microbial encapsulation with functionalizable silica shells[6]: silica encapsulation and thiol functionalization were simultaneously achieved, and chemical/biological functionalities were subsequently introduced by the thiol–maleimide coupling reaction.

Although the long-term cytopreservation and surface functionalization, by coating individual cells with robust materials, would be unquestionably beneficial for utilizing mammalian cells in the biotechnological and biomedical fields such as cell therapy, cell implantation, and cell-based sensors, the chemical manipulation of mammalian cells remained intractable because of the physicochemical fragility of cell membranes.[7] Unlike the unicellular microbial cells that possess robust cell walls composed of polysaccharides and peptidoglycans,[8] mammalian cells are enclosed in a weak membrane of lipid bilayers, which is fluidic and susceptible to environmental changes. Very recently, we suggested a bioinspired approach to the silica coating of individual HeLa cells.[9] Thiol functionalization of HeLa cells was achieved by the silica coating, and, more importantly, the silica-coated HeLa cells showed the enhanced tolerance against foreign aggressors such as trypsin and poly(allylamine hydrochloride). In this work, we investigate how reaction materials and conditions affect the viability of HeLa cells in the course of silica coating and optimize the silicification conditions for cytocompatible silica coating of HeLa cells. The manipulation of mammalian cells required the stringent selection of materials and processes, and the coating processes were screened by thoroughly testing the cytotoxicity of reaction media, cationic polymers, and silicic acid derivatives.

Microbial yeast cells have previously been encapsulated with silica in a bioinspired, cytocompatible fashion by two different methods (Table 1): (1) Polydiallyldimethylammonium chloride (PDADMAC) was used as a catalytic template for bioinspired silicification, based on the reports that PDADMAC was catalytically active for silica formation under physiologically mild conditions.[4c,f,10] After coating the individual yeast cells with PDADMAC by a layer-by-layer (LbL) technique, the resulting PDADMAC-coated yeast cells were immersed for 30 min in a phosphate-buffered solution (pH 5.5) of 50 mM tetramethyl orthosilicate (TMOS), which led to the formation of silica-encapsulated yeast (yeast@SiO_2). (2) Poly(ethyleneimine) (PEI) was used as a catalytic template. The PEI-coated yeast cells were immersed for 30 min in a phosphate-buffered solution (pH 7.4) of hydrolyzed TMOS (100 mM) and (3-mercaptopropyl)trimethyoxysilane (MPTMS, 100 mM), resulting in the formation of thiol-functionalized silica-encapsulated yeast (yeast@SiO_2^{SH}).

Table 1. Two different silicification conditions for individual encapsulation of microbial cells with silica.

	Precursor	Catalytic template	pH
Method 1[5]	TMOS	PDADMAC	5.5
Method 2[6]	TMOS and MPTMS	PEI	7.4

We investigated the cytocompatibility of the two previously reported conditions (pH values and identities of polymers and silica precursors) to HeLa cells by three independent assays (MTT, WST-1, and Live/Dead staining). MTT or WST-1, which is reduced from tetrazolium to a formazan derivative in metabolically intact cells, was used to quantify the cell viability by measuring the absorbance of the formazan derivative at a specific wavelength. The Live/Dead staining assay kit is composed of two fluorescent dyes that distinguish live and dead cells: Calcein AM is cleaved by esterase in a live cell to emit green fluorescence, while ethidium homodimer-1 (EthD-1) binds to the nuclei to emit red fluorescence in a dead or dying cell.

The MTT and WST-1 assays indicated that most HeLa cells survived both at pH 5.5 and at 7.4 after 30-min incubation (Figure 1), supported by the Live/Dead staining assay showing the green fluorescence at both pH values (Figure 2(a) and (b)). However, a closer look at the cellular structures in the Live/Dead staining images showed altered cellular morphologies at pH 5.5, presumably due to damage in the acidic environments. The toxicity of the catalytic templates (PDADMAC and PEI) was then tested with reduced incubation time to 10 min from 30 min. The cell viability was measured to be 2.26% (MTT) or 5.51% (WST-1) for PDADMAC in PBS (pH 5.5) and 26.4% (MTT) or 30.1% (WST-1) for PEI in PBS (pH 7.4), showing that PEI at pH 7.4 was far more cytocompatible than PDADMAC at pH 5.5. The cellular morphology was also not altered significantly in the PEI solution [Figure 2(c) and (d)]. On the same line, the mixture of TMOS and MPTMS (50 mM; 3:1 molar ratio) in PBS (pH 7.4) was more cytocompatible than 50 mM TMOS in PBS (pH 5.5): after 20-min incubation, the viability was 1.15% (MTT) or 5.49% (WST-1) for TMOS, and 19.6% (MTT) or 24.3% (WST-1) for the TMOS–MPTMS mixture. The low viability was also confirmed by a predominant emission of red fluorescence in the Live/Dead staining assay and cellular deformations including detachment and aggregation (Figure 2(e) and (f)). Collectively, the silicification method, which used PEI and the mixture of TMOS and MPTMS at pH 7.4, was more favourable than the other silicification method, although the observed cell viability was not sufficiently good enough for silica coating of HeLa cells.

We thought that it would be beneficial in mitigating the cytotoxicity of the catalytic polymers and silica precursors to add the ingredients essential for survival of mammalian

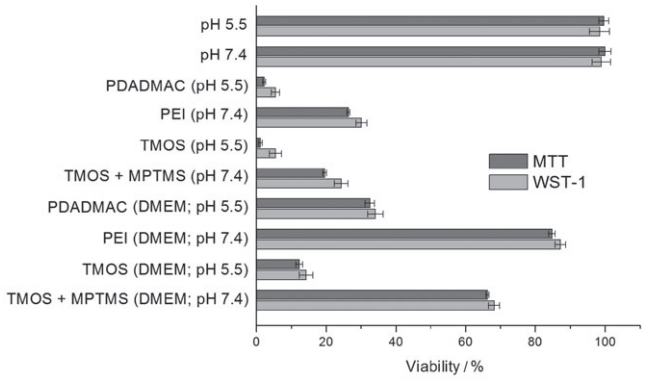

Figure 1. Viability of HeLa cells after treatment of various solutions. The viability was measured by MTT and WST-1.

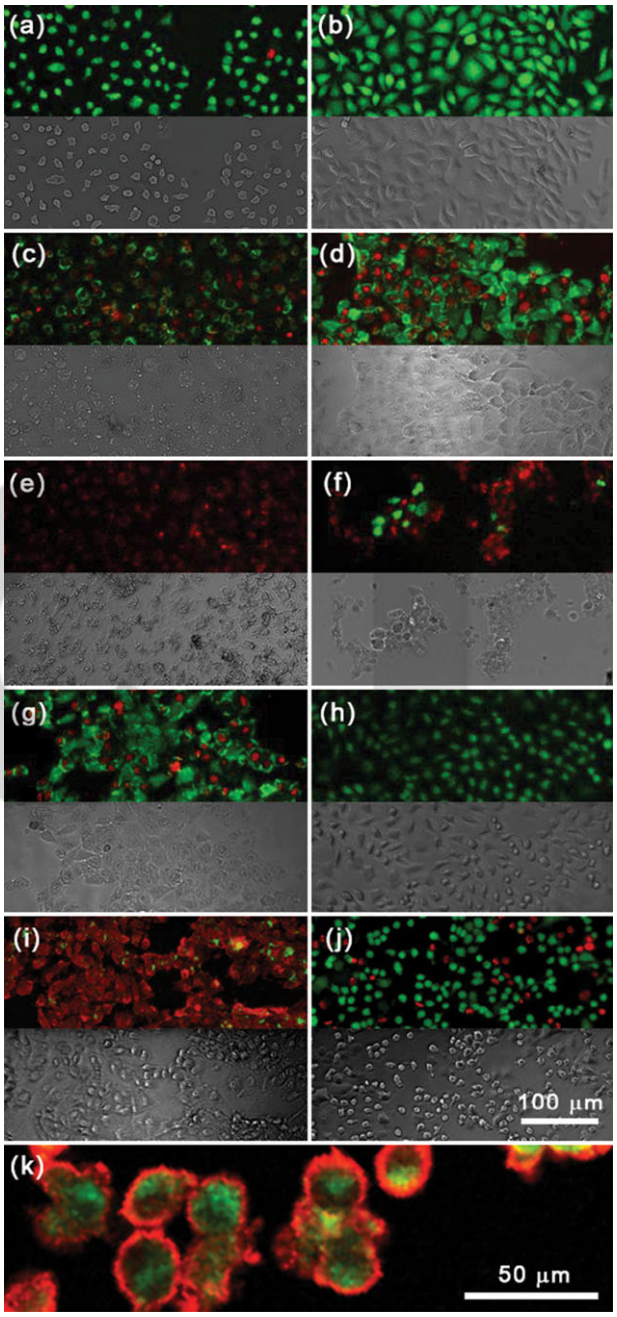

Figure 2. Fluorescence and optical images of HeLa cells. Living cells emit green fluorescence, and dead cells emit red fluorescence: (a) PBS at pH 5.5; (b) PBS at pH 7.4; (c) PDADMAC in PBS at pH 5.5; (d) PEI in PBS at pH 7.4; (e) TMOS in PBS at pH 5.5; (f) TMOS and MPTMS in PBS at pH 7.4; (g) PDADMAC in DMEM at pH 5.5; (h) PEI in DMEM at pH 7.4; (i) TMOS in DMEM at pH 5.5; (j) TMOS and MPTMS in DMEM at pH 7.4. (k) LSCM image of HeLa@SiO_2 after Live/Dead assay.

cells to the reaction medium. Therefore, we replaced PBS with Dulbecco's modified Eagle Medium (DMEM) buffer, which has been used widely as a culture medium and contains the ingredients necessary for survival and growth of HeLa cells. The cell viability was increased by this simple change of reaction media: 84.8% from 26.4% (MTT) or 87.2% from 30.1% (WST-1) for PEI in DMEM at pH 7.4. The Live/Dead staining images showed no red fluorescence, clearly indicating high cytocompatibility (Figure 2(g) and (h)). The cytocompatiblity of the TMOS–MPTMS mixture at pH 7.4 was also increased reasonably [viability: 66.4% from 19.6% (MTT) or 68.2% from 24.3% (WST-1)] (Figure 2(i) and (j)).

The reaction protocol (PEI and the TMOS–MPTMS mixture in DMEM at pH 7.4) was applied to individual coating of HeLa cells. Taking the fragility of mammalian cells into account, we also changed other reaction parameters from the previously reported ones.[6] PEI had been introduced to the microbial cell surface by repeated LbL processes, but in this work PEI was introduced to the cell membrane by one-step priming. Additionally, the PEI concentration was reduced to 0.1 mg/mL from 0.5 mg/mL, and the concentration of the silicic acid derivative to 50 mM from 100 mM. The reaction time was also shortened to 20 min from 30 min. Briefly, PEI was introduced to the HeLa cells detached from a culture flask by placing the cells in a PEI solution (0.1 mg/mL in DMEM) for 10 min. Then, the PEI-primed HeLa cells were immersed for 20 min in a silicic acid derivative solution, which was prepared by adding the hydrolyzed aqueous solutions of TMOS (1 M) and MPTMS (1 M) to DMEM (pH 7.4) in 1:3:76 (v/v/v) ratio, resulting in the formation of silica-coated HeLa cells (HeLa@SiO_2).

The viability of HeLa@SiO_2 was measured to be 76.8% (MTT) or 79.8% (WST-1) after silica coating, while the viability of native HeLa cells was 90.4% (MTT) or 95.6% (WST-1) after performing the same processes without PEI and silica precursors (Figure 3). Interestingly, the observed viability was higher than that after incubation in the silica precursors [66.4% (MTT) or 68.2% (WST-1)]. We thought that the PEI coating acted as a physical barrier to the penetration of silicic acid derivatives through the cell membrane and increased the cell viability.

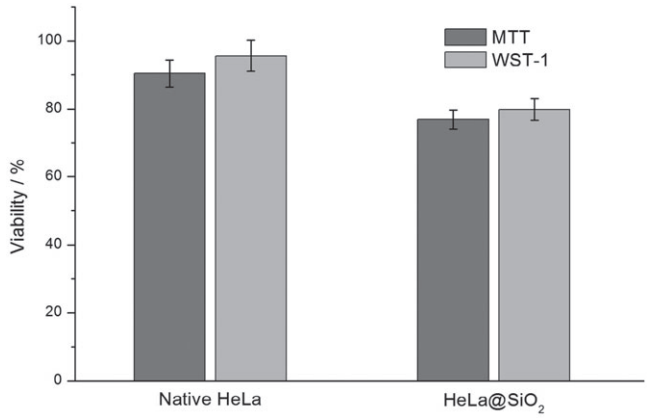

Figure 3. Viability of HeLa cells after silica coating. Native HeLa cells were treated with the same coating processes in the absence of PEI and silicic acid. The results of the MTT assay have been reported before.[9]

We also investigated the viability of HeLa cells by Live/Dead staining assay. Interestingly, the laser-scanning confocal microscopy (LSCM) image showed that the cytoplasm of the HeLa cell was stained in green and the surface of the cell in red [Figure 2(k)]. In the Live/Dead staining assay, the red-fluorescent dye, EthD-1, binds to nucleic acids after penetrating through the cell membrane, indicating whether the cells are damaged or dead. In our case, the ring-shaped red fluorescence implied that EthD-1 stained the silica coating, presumably because the penetration of EthD-1 was prevented by the strong electrostatic interactions between positively charged EthD-1 and negatively charged silica. The protective capability of the silica coating against positively charged materials has also been reported[9]: the silica-coated HeLa cells were protected effectively by the attack of polyallylamine hydrochloride (PAH). Taken together, the results implied that the chemical coating of mammalian cells would lead to enhanced cytoprotection, which is crucial for the development of many cell-based applications in biotechnology.

In summary, we optimized the silica-coating conditions for mammalian cells by screening various silicification conditions including catalytic templates, silicic acid derivatives, and pH. By carrying out all the processes in a culture medium, we successfully coated individual HeLa cells with silica in a cytocompatible fashion. We believe that the conditions described in this work could be optimized further for more labile mammalian cells, such as stem cells and immune cells, the cytoprotection and preservation of which would contribute greatly to biotechnological and medical areas.

Experimental

Materials. DMEM (Welgene, Gyeongsan, Korea), fetal bovine serum (FBS, Welgene, Gyeongsan, Korea), penicillin–streptomycin (5000 U/mL of penicillin and 5000 μg/mL of streptomycin, Welgene, Gyeongsan, Korea), phosphate-buffered saline solution (PBS, 10 mM, pH 7.4, Welgene, Gyeongsan, Korea), trypsin–ethylene diaminetetraacetic acid solution (1×, 0.05% trypsin, 0.53 mM EDTA·4Na in HBSS, Welgene, Gyeongsan, Korea), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA), water-soluble tetrazolium salt (WST-1, Abcam, Hanam, Korea), dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA), polydiallyldimethylammonium chloride (PDADMAC, average M_W: 100 000–200 000, 20 wt% in H_2O, Sigma-Aldrich, St. Louis, MO, USA), poly(ethyleneimine) (PEI, average M_n: ~10 000 by GPC, average M_w: ~25 000 by LS, Sigma-Aldrich, St. Louis, MO, USA), tetramethyl orthosilicate (TMOS, 99%, Sigma-Aldrich, St. Louis, MO, USA), (3-mercaptopropyl)trimethoxysilane (MPTMS, 95%, Sigma-Aldrich, St. Louis, MO, USA), and

Live/Dead viability/cytotoxicity kit (Life Technologies, Seoul, Korea) were used as received. Ultrapure water (18.3 MΩ/cm) from the Human Ultrapure System (Human Corp., Seoul, Korea) was used.

Cell Culture. HeLa cells were seeded in a cell culture flask with 10 mL of the DMEM solution containing 10% FBS and 1% penicillin–streptomycin, and the cells were incubated at 37 °C under a humidified atmosphere of 5% CO_2. When the cells were grown to 80% confluency, 2 mL of trypsin was added to the culture flask, and the cells were incubated at 37 °C for 5 min. After the cells were detached from the flask, 3 mL of DMEM was added, and the cells were collected by centrifugation and washed twice with a PBS solution.

Toxicity Test. After detaching HeLa cells from a cell culture flask by trypsinization, the cells were seeded in a 96-well dish with a density of 2.0×10^4 cells/mL. After a 12-h incubation, the prepared solutions were added to each well for viability test. The PBS buffer and serum-free DMEM solutions of polyamine (0.1 mg/mL) and silica precursors (50 mM) were prepared for toxicity test. The viability was investigated by three independent assays (MTT, WST-1, and Live/Dead staining).

MTT and WST-1 Assay. In a 96-well plate, the cells were seeded in 100 μL of DMEM with a density of 2×10^4 cells/mL. Ten microliters of MTT or WST-1 solution (5 mg/mL in PBS) was added to each well, and the cells were incubated for 4 h. For WST-1, the absorbance at 440 nm was measured with a microplate reader (Gemini EM, Molecular Devices, Sunnyvale, CA, USA). For MTT, the stained cells were collected by centrifugation, and DMSO was added to dissolve the water-insoluble purple formazan product. The absorbance at 560 nm was measured with a microplate reader.

Live/Dead Assay. The Live/Dead staining solution was prepared by dissolving 5 μL of Calcein AM and 20 μL of Ethd-1 in 10 mL of PBS buffer (50 mM; pH 7.4). The resulting solution was added to each well, and the cells were incubated for 20 min. After incubation, the stained cells were characterized with a fluorescence microscope (Eclipse Ti, Nikon, Melville, USA) or a confocal microscope (LSM 700 META, Carl Zeiss, Oberkochen, Germany).

Silica Coating. The HeLa cells were detached by trypsin from the culture flask and then collected by centrifugation. The cells were incubated in a serum-free DMEM solution of 0.1 mg/mL PEI (pH 7.4) for 10 min. After PEI priming, the cells were placed in the silicic acid derivative solution that had been prepared by hydrolyzing TMOS (1 M) and MPTMS (1 M) in 1 mM of an aqueous HCl solution for 20 min and adding the resulting solution of TMOS and MPTMS to a serum-free DMEM (pH 7.4) with 1:3:76 (v/v/v) ratio. After 20 min, the silica-coated HeLa cells were collected by centrifugation.

Characterization. Scanning electron microscopy (SEM) imaging was performed with a Sirion FEI XL FEG/SFEG microscope (FEI Co., Hillsboro, OR, USA) with an accelerating voltage of 10 kV after sputter-coating with platinum.

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References

1. P. Behrens, E. Bäuerlein Eds., *Handbook of Biomineralization: Biomimetic and Bioinspired Chemistry*, Vol. 2, Wiley-VCH, Weinheim, 2007.
2. (a) M. Sumper, E. Brunner, *Adv. Funct. Mater.* **2006**, *16*, 17; (b) M. Sumper, *Science* **2002**, *295*, 2430; (c) N. Kröger, R. Deutzmann, M. Sumper, *Science* **1999**, *286*, 1129.
3. M. Sumper, S. Lerenz, E. Brunner, *Angew. Chem. Int. Ed.* **2003**, *42*, 5192.
4. (a) S. H. Yang, *Solid State Sci.* **2013**, *23*, 1; (b) F. Nudelman, N. A. J. M. Sommerdijk, *Angew. Chem. Int. Ed.* **2012**, *51*, 6582; (c) S. H. Yang, J. H. Park, W. K. Cho, H.-S. Lee, I. S. Choi, *Small* **2009**, *5*, 1947; (d) M. Hildebrand, *Chem. Rev.* **2008**, *108*, 4855; (e) S. H. Yang, I. S. Choi, *Chem. Asian. J.* **2009**, *4*, 382; (f) W. K. Cho, S. M. Kang, D. J. Kim, S. H. Yang, I. S. Choi, *Langmuir* **2006**, *22*, 11208; (g) E. G. Bellomo, T. J. Deming, *J. Am. Chem. Soc.* **2006**, *128*, 2276; (h) D. J. Kim, K. B. Lee, T. G. Lee, H. K. Shon, W. J. Kim, H. J. Paik, I. S. Choi, *Small* **2005**, *1*, 992; (i) E. Brunner, K. Lutz, M. Sumper, *Phys. Chem. Chem. Phys.* **2004**, *6*, 854.
5. S. H. Yang, K.-B. Lee, B. Kong, J.-H. Kim, H.-S. Kim, I. S. Choi, *Angew. Chem. Int. Ed.* **2009**, *48*, 9160.
6. S. H. Yang, E. H. Ko, Y. H. Jung, I. S. Choi, *Angew. Chem. Int. Ed.* **2011**, *50*, 6115.
7. (a) D. Hong, M. Park, S. H. Yang, J. Lee, Y.-G. Kim, I. S. Choi, *Trends Biotechnol.* **2013**, *31*, 442; (b) E. Michelini, L. Cevenini, M. M. Calabretta, S. Spinozzi, C. Camborata, A. Roda, *Anal. Bioanal. Chem.* **2013**, *405*, 6155.
8. K. H. Schleifer, O. Kandler, *Bacteriol. Rev.* **1972**, *36*, 407.
9. J. Lee, J. Choi, J. H. Park, M.-H. Kim, D. Hong, H. Cho, S. H. Yang, I. S. Choi, *Angew. Chem. Int. Ed.* **2014**, *53*, 8056.
10. (a) J. Yuan, O. O. Mykharlyk, A. J. Ryan, S. P. Armes, *J. Am. Chem. Soc.* **2007**, *129*, 1717; (b) Y. Jia, G. M. Gray, J. N. Hay, Y. Li, G.-F. Unali, F. L. Baines, S. P. Armes, *J. Mater. Chem.* **2005**, *15*, 2002.

