Cell Coating

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Cytoprotective Silica Coating of Individual Mammalian Cells through Bioinspired Silicification**

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Dedicated to Professor George M. Whitesides on the occasion of his 75th birthday

Abstract: *The cytoprotective coating of physicochemically labile mammalian cells with a durable material has potential applications in cell-based sensors, cell therapy, and regenerative medicine, as well as providing a platform for fundamental single-cell studies in cell biology. In this work, HeLa cells in suspension were individually coated with silica in a cytocompatible fashion through bioinspired silicification. The silica coating greatly enhanced the resistance of the HeLa cells to enzymatic attack by trypsin and the toxic compound poly(allylamine hydrochloride), while suppressing cell division in a controlled fashion. This bioinspired cytocompatible strategy for single-cell coating was also applied to NIH 3T3 fibroblasts and Jurkat cells.*

In nature, most microbial cells have a tough coat that protects them from the environment.[1] Bacteria and fungi, for instance, are covered by cell walls composed of peptidoglycan and chitin, respectively.[2] Certain unicellular organisms, such as diatoms, Foraminifera, and coccolithophores, are encased by exoskeletal shells of silica (SiO_2) or calcium carbonate ($CaCO_3$).[3] Mammalian cells, however, do not have a robust cell wall or exoskeleton but are enclosed in a weak membrane consisting of a lipid bilayer, which is fluidic and susceptible to environmental changes. The mechanical fragility of mammalian cells makes it more daunting to chemically manipulate them than microbial cells because the in vitro treatment of mammalian cells requires the rigorous selection of materials and processes that ensure cell viability. In this respect, the development of a cytocompatible method for coating individual mammalian cells with mechanically durable materials would be beneficial for their practical use in biomedical fields and applications, including cell therapy, cell-based sensors, and cells-on-a-chip, which require the long-term protection and preservation of living cells, as well as in single-cell studies.[4]

The formation of siliceous cell walls in diatoms[5] has inspired researchers to mimic and apply the biosilicification processes to single-cell coating, by taking advantage of the mild reaction conditions involved and the chemical toughness of minerals, for protecting the cell from harmful environments and manipulating cellular activities at a single-cell level.[6] Silica shells were formed on individual microbial cells, such as *Saccharomyces cerevisiae* and *Synechocystis*,[7] and the bioinspired silicification approach also has been expanded to the abiological titania (TiO_2) or TiO_2–SiO_2 coating of *Chlorella* and other microbes.[8] The coated microorganisms showed increased resistance to external stressors: for example, *Chlorella* showed threefold enhanced tolerance to thermal stress when coated with the TiO_2–SiO_2 hybrid.[8a] Herein, we report the first example of the cytocompatible and cytoprotective nanocoating of mammalian cells with mechanically durable silica. Mammalian cells (HeLa cells, NIH 3T3 fibroblasts, and Jurkat cells) were individually coated with silica through bioinspired silicification (Figure 1). The silica coat effectively protected the HeLa cells against proteolytic attack by trypsin and the toxic compound poly(allylamine

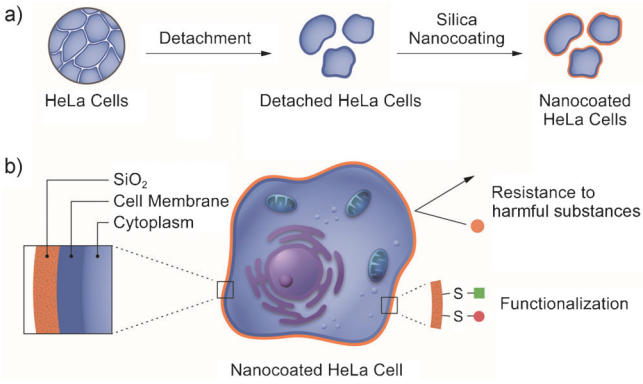

Figure 1. a) A schematic representation of the silica nanocoating of HeLa cells through bioinspired silicification, which consists of 10 min priming with PEI and a subsequent 20 min silicification in serum-free DMEM medium. b) The silica nanocoat protects the encased HeLa cells against enzymatic attack by trypsin and chemical attack by PAH, as well as providing an opportunity for post-functionalization and controlling cell division while maintaining viability.

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hydrochloride). In addition, the silica nanocoat suppressed cell growth while providing the adhesion-dependent HeLa cells with a survival scaffold.

In bioinspired silicification, polyamines including poly(ethyleneimine) (PEI) have been used as a catalytic template for silicification, where specific interactions between polyamines and silicic acid lead to in vitro silica formation under mild conditions.[5,9] In particular, relatively thick silica films were formed from a mixture of tetramethyl orthosilicate (TMOS) and 3-mercaptopropyl trimethoxysilane (MPTMS) in the presence of PEI in a short period of time.[10] The zeta potential of HeLa cells was reported to be about −20 mV[11] and PEI could be deposited onto HeLa cells electrostatically.[12] In addition, PEI, a nonviral vector that has been intensively investigated for gene therapy,[13] was found to be reasonably cytocompatible in our system. After detaching the HeLa cells from the culture flask and priming themwith PEI (0.1 mg mL^{-1}), we collected them by centrifugation, resuspended them in serum-free Dulbecco's modified Eagle medium (DMEM; pH 7.4) as a reaction medium, and investigated their viability by using the MTT colorimetric assay. The MTT assay, in which 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) is reduced to a formazan structure by NAD(P)H-dependent cellular oxidoreductases in metabolically intact cells, showed that the viability was 87.2% after 10 min PEI priming (Figure 2a). Following the introduction of PEI, silica coating was performed for 20 min in a DMEM solution of TMOS and MPTMS (1:3 (v/v); pH 7.4) to give silica-coated HeLa cells (HeLa@SiO_2 cells). The viability of the HeLa@SiO_2 cells was measured to be 76.8%: this viability value is noteworthy because the viability of *Saccharomyces cerevisiae*, which has a cell wall, was reported to be about 77% after silicification.[7c] As a reference, the viability of native HeLa cells was 90.3% after the same processes without PEI and the silica precursors. In this work, we used the relatively noncytotoxic PEI for introducing a catalytic template for the bioinspired silicification of HeLa cells, but the cell viability after silicification would be increased further by proper modifications to PEI, such as pegylation.[14]

The HeLa@SiO_2 cells were characterized by scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) spectroscopy, infrared (IR) spectroscopy, cell culture tests, and laser-scanning confocal microscopy (LSCM). The morphology of the HeLa@SiO_2 cells was investigated by SEM with native HeLa cells as a comparison (Figure 2b). Unfixed native HeLa cells were seriously damaged or ruptured under the sample preparation conditions (dehydration and reduced pressure; Figure S1 in the Supporting Information), while the HeLa@SiO_2 cells maintained their original round shape even without fixation. The magnified SEM image in Figure 2b indicates that the silica coat is composed of silica nanoparticles. The EDX spectrum of the HeLa@SiO_2 cells, which shows Si and S peaks at 1.74 and 2.35 keV, respectively, confirmed successful silica formation and also the incorporation of MPTMS into the silica coat. In the IR spectrum, we observed an intense peak corresponding to silica between 1000 and 1250 cm^{-1} (Si–O–Si asymmetric stretching; Figure S2).

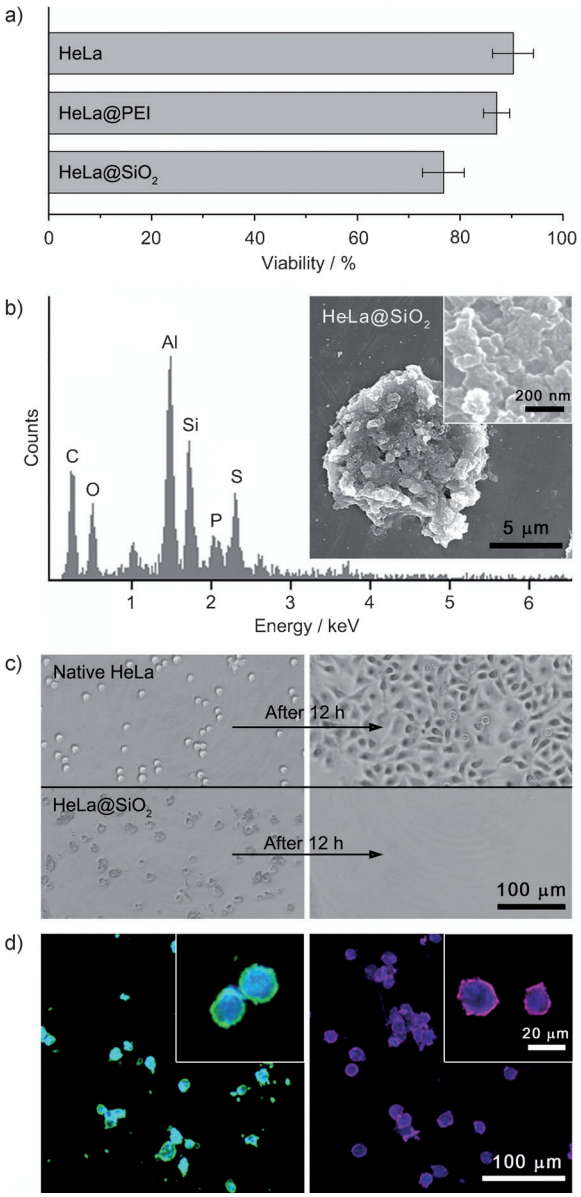

Figure 2. a) The viability of HeLa cells after PEI priming and silica coating. Native HeLa cells were subjected to the reaction conditions for both PEI priming and silica coating in the absence of PEI and silica precursors as a control. b) SEM micrographs and EDX spectrum of HeLa@SiO_2 cells. c) Optical micrographs of native HeLa cells (top) and HeLa@SiO_2 cells (bottom) in culture flasks right after cell seeding (left) and after 12 h culture and decanting of the culture medium (right). d) LSCM images of HeLa@SiO_2 cells after functionlization with *N*-(5-fluoresceinyl)maleimide (left) or with EZ-link maleimide-PEG_2-biotin and subsequent complex formation with TRITC-conjugated streptavidin (right). The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). DAPI staining is shown in blue; fluorescein in green; and TRITC in red.

The formation of a silica coat was also evidenced by the cell culture results. Native HeLa and HeLa@SiO_2 cells were incubated in a cell culture flask under standard culture conditions (37 °C, 5% CO_2) with the same seeding density (2.0×10^4 cells mL^{-1}). Upon incubation, the native HeLa cells adhered and grew flat on the surface; by contrast, the

HeLa@SiO_2 cells maintained their spherical shape without any observable attachment to the surface (Figure 2c). More importantly, the number of HeLa@SiO_2 cells remained unchanged after 12 h culture. Although the viability of the HeLa@SiO_2 cells decreased from 76.8% to 58.7%, the maintenance of both shape and number of HeLa@SiO_2 cells implies that the growth of the HeLa cells ceased or was at least suppressed by the silica coat. It could be that cell division is retarded by the mechanical toughness of the silica coat: a previous report showed that the cell cycle was regulated by the stiffness of hydrogel microbeads used to encapsulate HeLa cells.[15] These results imply that cell cycle progression could be controlled by simple chemical coating and tuned by varying the physicochemical properties of the coat. The HeLa@SiO_2 cells were recovered completely from the cell culture flask when the medium was decanted, thus showing that the mechanically durable silica coat was successfully formed and acted as a physical barrier to cell exposure to the outside environment.

The incorporation of MPTMS into the silica coat is also advantageous because the thiol (SH) group in MPTMS could be utilized for post-functionalization under cytocompatible conditions.[7b] This potential for post-functionalization would enable the applications of the HeLa@SiO_2 cells to cell-based sensors and cells-on-a-chip that would benefit from the site-specific localization of cells in the devices.[4] *N*-(5-Fluoresceinyl)maleimide or biotin-linked maleimide was coupled to the silica coat through 1,4-addition under mild conditions (phosphate-buffered saline, pH 7.4). The LSCM images show clear-cut green rings around the HeLa cells from fluorescein or red ones after complexation of the biotin with tetramethylrhodamine (TRITC)-linked streptavidin, thus indicating uniform coat formation (Figure 2d). Taken together, these results confirmed the highly cytocompatible coating of individual HeLa cells with silica through bioinspired silicification.

In addition to the mechanical rigidity given by the silica coat, the HeLa@SiO_2 cells acquired greatly enhanced resistance to proteolytic attack by trypsin. Trypsin is highly cytotoxic to mammalian cells owing to its nonspecific proteolytic activity,[16] although it is used with short exposure times for the detachment of adherent cells. For example, when native HeLa cells, after detachment from a culture flask, were incubated in a solution of trypsin and ethylenediaminetetraacetic acid (EDTA), the viability decreased sharply: the survival ratio was 0.325 after 2 h incubation, and no cells were alive after 4 h (Figure 3a). In stark contrast, 73.3% of the HeLa@SiO_2 cells were still viable after 2 h under the same conditions, and more than one third of the HeLa@SiO_2 cells survived a 4 h treatment. We believe that this surprising resistance results from precluded access of trypsin to the HeLa cells inside the silica coat. We also tested the cytoprotective ability of the silica coat against poly(allylamine hydrochloride) (PAH) because PAH, which has primary amines, was reported to be highly cytotoxic to cells, including microbes with cell walls.[17] Native HeLa and HeLa@SiO_2 cells were incubated in a phosphate-buffered saline (PBS) solution of PAH with various concentrations for 1 h. The cytoprotection of the silica coat against PAH was noteworthy (Figure 3b): for example, after 1 h exposure to PAH

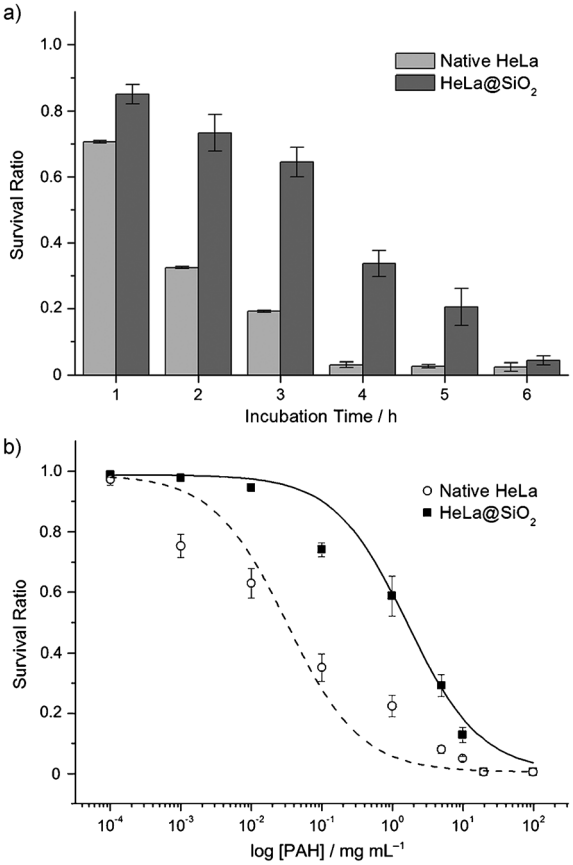

Figure 3. a) Enhanced resistance of HeLa@SiO_2 cells to enzymatic attack by trypsin. The trypsin solution was prepared by diluting the trypsin-EDTA solution (0.05% of trypsin and 0.53 mM of EDTA in HBSS) with Hank's balanced salt solution (HBSS) (v/v = 1:1). b) Graphs of survival ratio versus concentration of PAH in PBS buffer (pH 7.4) for native HeLa cells and HeLa@SiO_2 cells. The cells were incubated for 1 h at 37°C.

(1 mg mL^{-1}), about 80% of the native HeLa cells were dead (survival ratio: 0.199), whereas 58.2% of the HeLa@SiO_2 cells survived. The IC_{50} value, the concentration of PAH at which the survival ratio reached 0.5, was calculated to be 32 mg L^{-1} for native HeLa cells. The silica coat effectively protected the HeLa cells inside and the IC_{50} value increased by two orders of magnitude to 1640 mg L^{-1} for HeLa@SiO_2 cells. The protection likely involves a charge-driven deposition of positively charged PAH onto the negatively charged silica, with the resulting charge compensation and/or reversal inhibiting the further adsorption and penetration of PAH, as in the layer-by-layer process.[18]

The same strategy was applied to NIH 3T3 fibroblasts and Jurkat cells to show its versatility in the silica coating of individual mammalian cells. NIH 3T3 fibroblasts are adherent cells that require a supporting scaffold for survival and growth. Jurkat cells are a sphere-shaped, immortalized cells derived from human T lymphocytes. Jurkat cells are considered to be a model for T cells, which, given their involvement in the immune responses in the body, would be a strong cell-therapy candidate for wound healing. After silica coating,

viability was measured to be 80.6% for fibroblasts and 50.8% for Jurkat cells (Figure S3).

In summary, we report a bioinspired, cytocompatible method for forming a mechanically durable silica coat on the surface of individual mammalian cells (HeLa, NIH 3T3, and Jurkat cells). The silica-coated HeLa@SiO_2 cells proved reasonably viable for short-term assays; the silica coat was not degradable, which might ultimately cause cell death. In this respect, the development of stimuli-responsive coats that degrade on-demand would facilitate advancement in the cell-coating field.[4b] In addition, HeLa@SiO_2 cells showed unprecedented resistance to the otherwise lethal trypsin and poly(allylamine hydrochloride). The observed protection was achieved simply by the physical impermeability of the silica coat but we believe that protections against other stressors, such as heat, UV irradiation, or desiccation, would be possible with further modification of the coat and/or incorporation of functional materials into the coat.

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