Thermoprotective Bioencapsulation of Chlorella Cells within Silica/Titania Nanoshells

Ko E. H., Yoon Y. and Choi I. S.*
Cell-Encapsulation Res & Molec-Level Interface Res Center, KAIST, Korea. (eunhyea@kaist.ac.kr)

INTRODUCTION

The cytocompatible bioencapsulation of individual living-cells within thin hard shells (<100 nm), such as silica (Yang 2009), would open the door to unforeseen arena in the aspect of control of cellular metabolism. The physically-stable but chemically-permeable shell encasing a single cell allows for control in cell cycles and protection against physical deformation and harsh chemical/biological conditions, on top of maintenance of viability.

Siliceous cells found in nature, such as diatom (Sumper 2002) and glass sponge (Aizenberg 2004), have inspired scientists to biomimetically apply the biosilicification processes occurring under physiological conditions. The biomimetic approach also provides methods for forming abiological minerals, such as titania (TiO_2), Ga_2O_3, ZrO_2, and $BaTiOF_4$, under mild conditions (Dickerson 2008), and has been applied to the individual encapsulation of *Chlorella* cells with TiO_2 (Yang 2012). Composites of biological SiO_2 and abiological TiO_2 have intensively been studied in material science (Zeleňák 2006). For example, thermo-oxidative stability of polystyrene was enhanced by addition of titanium-containing polyhedral oligomeric silsesquioxane (Carniato 2009). In the cell encapsulation, this hybrid material could effectively dissipate heat energy, therefore cells could tolerate high temperature. This novel property would give new route to protect marine microalga from El Niño as well as electric heating of cell-based biocircuit. Herein, we attempted to encapsulate *Chlorella* cells with a composite of biological SiO_2 and abiological TiO_2 in a single step, by utilizing a catalytic peptide for both oxides.

MATERIALS AND METHODS

First, we found that a 20-mer peptide, $(RKK)_4D_8$ (R: arginine; K: lysine; D: aspartic acid) could be used as a catalytic template for cytocompatible formation of silica/titania composites. Also, our previous report showed that the $(RKK)_4D_8$ peptide was cytocompatible with *Chlorella* (Yang 2012). We used a marine microalgae *Chlorella sp.* C-141 that had been sampled from surface water in the Jindong coast, Korea (Hur 2008). After depositing $(RKK)_4D_8$ onto the *Chlorella* surface by electrostatic interactions for 2 min, we added a mixture of the silica and titania precursors (silicic acid : titanium bis(ammonium lactato)dihydroxide; 1:1, molar ratio) to the suspension and incubated the mixture for 2 min. This cycle was repeated 3 times in total, leading to the formation of silica/titania-encapsulated *Chlorella* cells (*Chlorella*@SiO_2-TiO_2).

RESULTS AND DISCUSSION

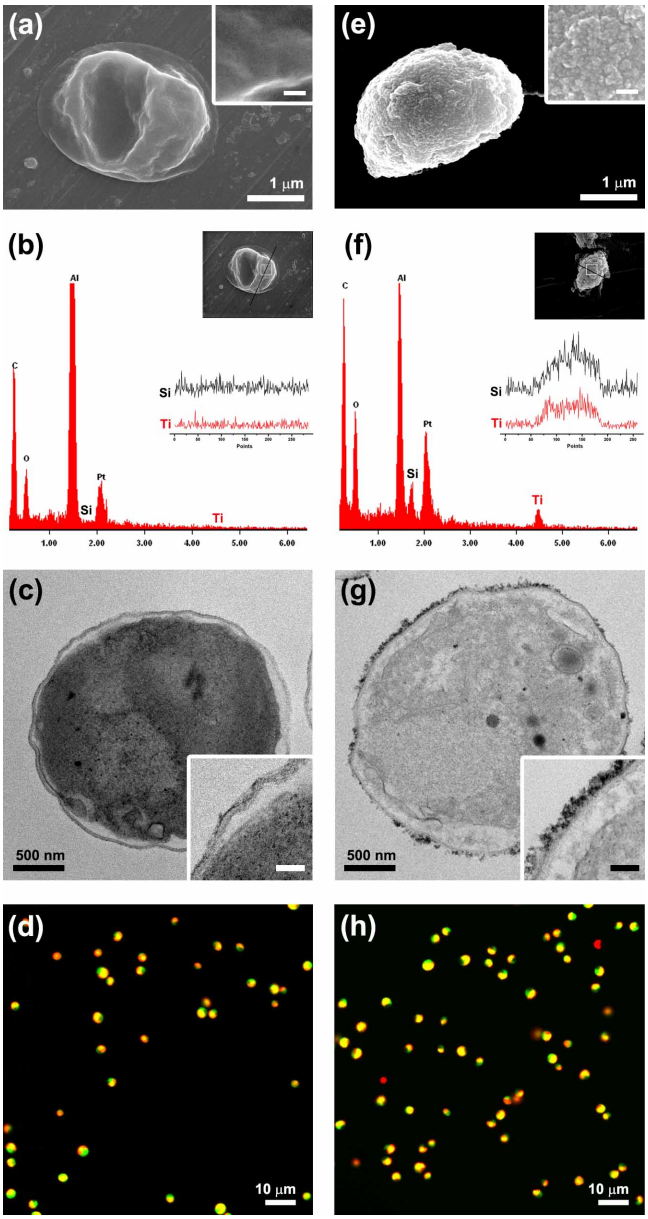

Figure 1 : (a-d) native *Chlorella*: SEM micrographs, EDX elemental analysis and Si and Ti line profile, TEM micrographs, and viability. (e-h) *Chlorella*@SiO_2-TiO_2: SEM micrographs, EDX elemental analysis and Si and Ti line profile, TEM micrographs, and viability. Inset figures in (a), (c), (e) and (g) show the surface morphologies of each *Chlorella* cells (scale bar: 100 nm). *Chlorella* cells in greenish and reddish yellow *Chlorella* cells are considered alive, and the red ones are considered dead.

The resulting *Chlorella*@SiO_2-TiO_2 was characterized by scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) spectroscopy, and transmission electron microscopy (TEM). All results confirmed that the *Chlorella* cells were individually encapsulated with silica/titania nanocomposites, and the shell thickness was about 50 nm (Figure 1).

After encapsulation, we used fluorescein diacetate (FDA) for the cell-viability test. FDA examines the activity of intracellular esterases and the membrane integrity, and emits green fluorescence by reactive oxygen species (Cronin 2004). The FDA test showed that the cell-viability was about 86% after 3-by-3 cycles (Figure 1h).

To identify the silica/titania nanocomposites that could shield at elevated temperature, the viability *Chlorella*@SiO_2-TiO_2 against thermal stress was investigated. First, thermal stress was given at 45 ^oC in a shaking water bath, while normal culture condition of *Chlorella sp.* C-141 cells was 23 ^oC in a shaking incubator (Hur 2008). After 2-h thermal treatment, most of native *Chlorella* cells were dead (18.1% viability). However, *Chlorella*@SiO_2-TiO_2 showed 54.3% viability, which was 3-fold enhanced, in comparison with viability of thermo-treated native *Chlorella* cells (2 h in Figure 2). To know how long the *Chlorella* cells could tolerate high temperature, the cells were treated from 1 h to 5 h at 45 ^oC. According to Figure 2, encapsulated cells could tolerate at elevated temperature up to 4 h, and after 4 h both encapsulated cells and native cells were mostly dead. We concluded that 3-by-3 deposited silica/titania shell can protect *Chlorella* from 4-h heat stress at a temperature of 45 ^oC.

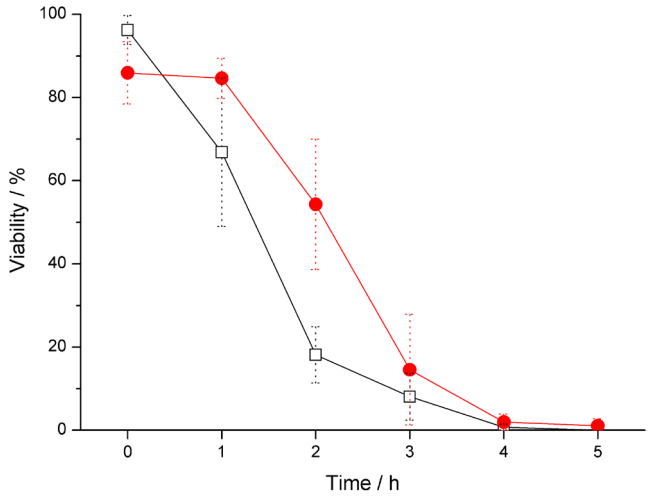

Figure 2 : Viability curves of native *Chlorella* (□), and *Chlorella*@SiO_2-TiO_2(●) during thermal treatment at 45 ^oC.

CONCLUSIONS

In conclusion, living *Chlorella* cells were individually encapsulated with a composite of biological SiO_2 and abiological TiO_2, which utilized a catalytic $(RKK)_4D_8$ peptide for both oxides. The formed silica/titania shell maintained the cell viability, protected against thermal stress. Of interest, the incorporation of SiO_2 to the shell increased the viability of the encapsulated *Chlorella* cells and the thickness of the artificial shell, compared with the TiO_2 shell itself. The synergistically emerging properties would be realized more in a designed fashion for other biological-nonbiological combinations made possible by bioinspired mineralization. Considering that many eggs are protected by the outmost inorganic shells and biological metabolism is controlled tightly by layered organic-inorganic shells, we believe that the formation of artificial inorganic-shells would be one of the promising approaches to the generation of artificial shields.

REFERENCES

- Aizenberg J. et al. (2004) *Biological glass fibers: Correlation between optical and structural properties*. Proc. Natl. Acad. Sci. USA 101(10) 3358-3363
- Carniato F. et al. (2009) *Titanosilsesquioxanes embedded in synthetic clay as a hybrid material for polymer science*. Angew. Chem. Int. Ed. 48(33) 6059-6061
- Cronin M. T. et al. (2004) *Assessment and modeling of the toxicity of organic chemicals to Chlorella vulgaris: development of a novel database*. Chem. Res. Toxicol. 17(4) 545-554
- Dickerson M. B. et al. (2008) *Protein- and Peptide-Directed Syntheses of Inorganic Materials*. Chem. Rev. 108(11) 4935-4978
- Hur S. B et al. (2008) *Korea Marine Microalgae Culture Center - List of Strains*. Algae 23(3) 1-68
- Sumper M. (2002) *A Phase Separation Model for the Nanopatterning of Diatom Biosilica*. Science 295(5564) 2430-2433
- Yang S. H. et al. (2009) *Biomimetic Encapsulation of Individual Cells with Silica*. Angew. Chem. Int. Ed. 48(48) 9160-9163
- Yang S. H. et al. (2012) *Cytocompatible encapsulation of individual Chlorella cells within titanium dioxide shells by a designed catalytic peptide*. Langmuir 28(4) 2151-2155
- Zeleňák V. et al. (2006) *Mesoporous Silica Modified with Titania: Structure and Thermal Stability*. Chem. Mater. 18(14) 3184–3191

ACKNOWLEDGMENTS

This work was supported by Basic Science Research Program (2012R1A3A2026403, 2012-0000908) and the National Junior Research Fellowship (NRF-2012 H1A8002548) through the National Research Foundation of Korea (NRF) and the R&E program of Korea Science Academy of KAIST.