RIKEN Seminar

Date: Thursday, 13th March 2014, 16:00-17:00
Venue: 2F Seminar room, Nanoscience building (Emergent Matter Science Research Laboratory)

Artificial Spores: Cytoprotective Nanoencapsulation of Individual Living Cells

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Introduction

Adaptability is crucial for survival in nature, and various organisms take extensive measures to minimize the stress of an unfavorable environment. Some species respond to harsh conditions in an extreme manner and form a physical enclosure to isolate and protect themselves completely from their surroundings. This extreme survival mechanism, called sporulation, is often observed in bacteria, fungi, and, in some rare cases, fish. Bacterial endospores, in particular, display a remarkable resistance to malnutrition, heat, radiation, chemicals, and desiccation

An emerging field of research has recently dedicated itself to emulating the protective properties of endospores.[1] By chemically encapsulating non-spore-forming cells individually in ultrathin, robust shells, virtually every cell can be endowed with the defensive capabilities that an endospore may possess. These new types of cell-in-shell structures are called 'artificial spores'. The fabrication of nanometric shells (<100 nm) for single living cells, however, is a delicate process. Of importance, the chemical construction of cytoprotective layers should not come at the cost of cell viability. Despite this obstacle, the field of artificial spores has progressed rapidly and has successfully engineered several impressive spore-like features (Figure 1). The most notable achievements so far include: (i) cell-division control – control over the growth or division of living cells, to some extent, by tuning the mechanical and physicochemical properties of nanometric shells; (ii) enhanced resistance to external stressors – increased tolerance against enzymatic aggression, chemical stressors, malnutrition, heat, and radiation, which enables the coated cells to outlive native, unprotected types; and (iii) chemical functionalizability – cytocompatible shell functionalization, useful for biotechnological applications that demand specialized recognition features.

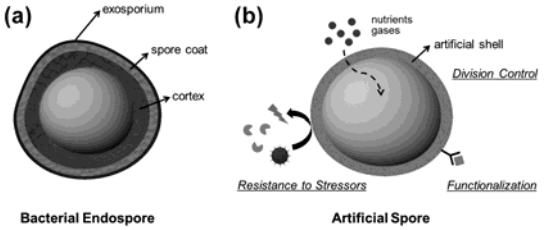

Figure 1. Structures of a bacterial endospore and artificial spore. (a) Endospore shell is composed of cortex, spore coat, and exosporium, which have important protective roles in harsh environments. (b) Cytoprotective nanoencapsulation of single living cells leads to artificial spores chemically endowed with cell-division control, enhanced resistance, and chemical functionalizability.

An Example: Resistance to Foreign Aggressions

Perhaps the most defining characteristic of natural spores is their remarkable ability to withstand otherwise lethal stressors. Artificial spores have been engineered to possess enhanced resistance to desiccation, high temperatures, radiation, and caustic species depending on the materials and methods used in shell synthesis. For example, yeast cells encapsulated individually in silica (yeast@SiO_2) and native, bare yeast showed different viabilities. After a 30-day incubation in pure water at 4 ^oC the survival rate of yeast@SiO_2 was three times higher than that of bare yeast (Figure 2a).[2a] Artificial shells have also shown increased durability against other types of stressors. Polydopamine-encapsulated yeast (yeast@PD) were fairly resistant to enzymatic attack by lyticase, a cell-wall-lysing enzyme complex,[2b] and the silica-titania (TiO_2) shell increased the thermotolerance of *Chlorella* cells after encapsulation (Figure 2b and c).[2c]

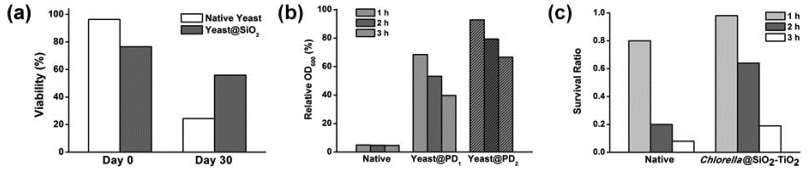

Figure 2. Resistance to foreign aggressions. (a) Enhanced resistance of yeast@SiO2 against malnutrition (in a pure water at 4 ^oC for 30 days). (b) Enhanced resistance of yeast@PD against lysticase.(c) Enhanced thermotolerance of *Chlorella*@SiO_2-TiO_2.

Conclusions and Future Direction

Currently available artificial spores have exhibited enhanced tolerance to external stressors, regularly alongside delayed cell division. What remains to be accomplished is a finer level of control to unlock more-sophisticated capabilities (Figure 3). Key traits that have yet to be incorporated include: (i) stimulus-responsive nanoshells – on-demand degradation in response to a specific external stimulus; (ii) multilayered shell formation – multiple layers, each having a different function; and (iii) nanoencapsulation of multicells – controlled encapsulation of a desired number of cells, ideal for studies in cell-to-cell communication, tissue engineering, and cell therapy. Artificial spores in their current state are somewhat rudimentary and demonstrate only the most fundamental qualities of endospores. When more-advanced, multifaceted, 'smart' shells are realized artificial spores will become freely designable platforms for a multitude of cell-based applications.

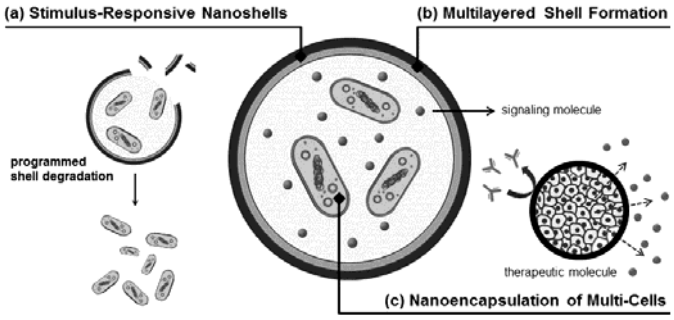

Figure 3. Suggested future direction for artificial spore research. (a) Stimulus-responsive nanoshells: degradation in response to a specific external signal for on-demand division control. (b) Multilayered shell formation: multiple layers with different, orthogonal functions. (c) Nanoencapsulation of multicells: encapsulation of the predetermined number of microbial cells for investigation of cell-to-cell communications and single-cell biology and encapsulation of mammalian cells for tissue engineering and cell therapy.

References

1. (a) S. H. Yang, D. Hong, J. Lee, E. H. Ko and I. S. Choi, *Small*, **9**, 178 (2013). (b) D. Hong, M. Park, S. H. Yang, J. Lee, Y.-G. Kim and I. S. Choi, *Trends Biotechnol.*, **31**, 442 (2013). (c) J. H. Park, S. H. Yang, J. Lee, E. H. Ko, D. Hong and I. S. Choi, *Adv. Mater.*, DOI: 10.1002/adma.201304568.
2. (a) S. H. Yang, K.-B. Lee, B. Kong, J.-H. Kim, H.-S. Kim and I. S. Choi, *Angew. Chem. Int. Ed.*, **48**, 9160 (2009). (b) S. H. Yang, S. M. Kang, K.-B. Lee, T. D. Chung, H. Lee, and I. S. Choi, *J. Am. Chem. Soc.*, **133**, 2795 (2011). (c) E. H. Ko, Y. Yoon, J. H. Park, S. H. Yang, D. Hong, K.-B. Lee, H. K. Shon, T. G. Lee and I. S. Choi, *Angew. Chem. Int. Ed.*, **52**, 12279 (2013).