Cytoprotection

DOI: 10.1002/anie.201408454

Cytoprotective Alginate/Polydopamine Core/Shell Microcapsules in Microbial Encapsulation**

Beom Jin Kim, Taegyun Park, Hee Chul Moon, So-Young Park, Daewha Hong, Eun Hyea Ko, Ji Yup Kim, Jong Wook Hong, Sang Woo Han, Yang-Gyun Kim, and Insung S. Choi**

Abstract: *Chemical encapsulation of microbes in three-dimensional polymeric microcapsules promises various applications, such as cell therapy and biosensors, and provides a basic platform for studying microbial communications. However, the cytoprotection of microbes in the microcapsules against external aggressors has been a major challenge in the field of microbial microencapsulation, because ionotropic hydrogels widely used for microencapsulation swell uncontrollably, and are physicochemically labile. Herein, we developed a simple polydopamine coating for obtaining cytoprotective capability of the alginate capsule that encapsulated Saccharomyces cerevisiae. The resulting alginate/polydopamine core/shell capsule was mechanically tough, prevented gel swelling and cell leakage, and increased resistance against enzymatic attack and UV-C irradiation. We believe that this multifunctional core/shell structure will provide a practical tool for manipulating microorganisms inside the microcapsules.*

Cell microencapsulation, the confinement of living cells (e.g., therapeutic pancreatic islets and microbial probiotics) in 3D microcapsules, has potential for applications in cellular medicine,[1] probiotics packaging,[2] biomass production,[3] and biosensors,[4] as well as providing a research platform for investigating cell-to-cell communications such as quorum sensing.[5] Cell-laden microcapsules with different sizes have been fabricated using various techniques including droplet-based microfluidics,[6] because a specific range of capsule sizes is preferred in the applications, especially in cellular medicine and biosensors. Ideally, the cells in the capsule are protected from harmful external aggressors and they survive and are biologically functional. Since the temporal immunoprotection and potential therapeutic functioning of pancreatic islets in vivo were reported,[7] various methods have been attempted to achieve long-term cytoprotection of therapeutic cells, including layer-by-layer coating,[8] microparticles-in-capsule formation,[9] and two-fluid coaxial electro-jetting.[10]

The cytoprotection of microbial cells inside capsules is vital because the encapsulated microbes face a variety of harmful conditions, such as exposure to toxic chemicals and enzymes, UV radiation, and extreme pH values and temperatures, both in vivo and in vitro.[11] For example, the microbes should be protected and preserved under everyday conditions to prolong the shelf life for such applications as biosensors, biocatalysis, and biomass. Compared with the therapeutic cells that generally do not grow, microbial growth can also cause unwanted outcomes because the uncontrolled growth can lead to capsule rupture and microbial exposure to the outside. Microbe leakage has been particularly problematic because ionotropic hydrogels (e.g., alginate) that are commonly used for cell microencapsulation are mechanically fragile and chemically labile.[12] To overcome these problems, the use of stress-tolerant and growth-suppressed biofilm-like microbes was suggested for microbial encapsulation.[13] Another approach has been the coating of ionotropic microbeads. In this case, chitosan coats were formed electrostatically on alginate beads containing *Lactococcus lactis* ssp. *cremoris* and reduced cell leakage was detected.[14] However, chitosan is highly lethal to many microorganisms including fungi, algae, and some bacteria.[15] Additionally, the chitosan coat was mechanically fragile because it was formed by electrostatic interactions between cationic chitosan and anionic alginate. Therefore, a cytocompatible and mechanically robust coating is highly desired for the prevention of cell leakage and the effective protection of the cells inside the capsule. In this paper, we present a one-step, cytocompatible polydopamine (PD) coating[16] to generate alginate/PD core/shell microcapsules that encapsulate yeast *Saccharomyces cerevisiae* cells. The covalently linked PD shell is mechanically durable and prevents gel swelling. More importantly, the core/shell structures enhance resistance against external stresses, such as enzymatic attack and UV-C irradiation, as well as preventing cell growth and leakage.

S. cerevisiae cells expressing GFPuv (GFPuv-yeast), which emit green fluorescence when they are exposed to $\lambda = 405$ nm light, were encapsulated within Ca^{2+}-alginate beads 192.2 μm in diameter (coefficient of variation $< 1.9\%$) by electrohydrodynamic jetting (Figure 1; for the experimental details including the construction of GFPuv-yeast, see the Supporting Information). The as-prepared beads were then coated with PD simply by shaking the beads in a Tris-HCl buffer solution of dopamine hydrochloride (5 mg mL^{-1}) at room temperature for 12 hours, resulting in

[*] B. J. Kim, T. Park, H. C. Moon, D. Hong, E. H. Ko, J. Y. Kim, J. W. Hong, Prof. S. W. Han, Prof. I. S. Choi
Center for Cell-Encapsulation Research
Department of Chemistry, KAIST, Daejeon 305-701 (Korea)
E-mail: ischoi@kaist.ac.kr
S.-Y. Park, Prof. Y.-G. Kim
Department of Chemistry, Sungkyunkwan University
Suwon 440-746 (Korea)
E-mail: ygkimmit@skku.edu

[**] This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (MSIP) (2012R1A3A2026403).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201408454.

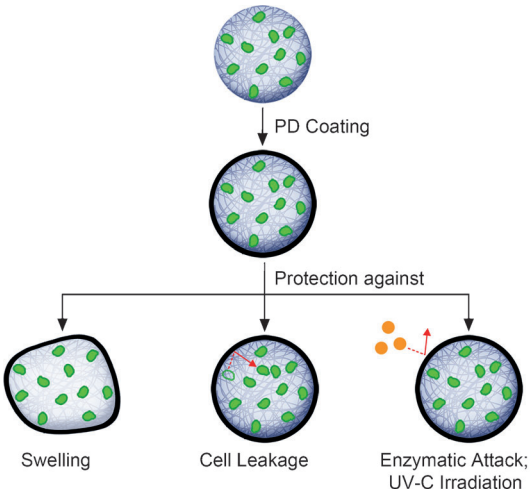

Figure 1. Cytoprotective polydopamine (PD) coating of cell-laden alginate microbeads. Alginate microbeads are represented by the blue spheres; yeast cells are represented by the green circles.

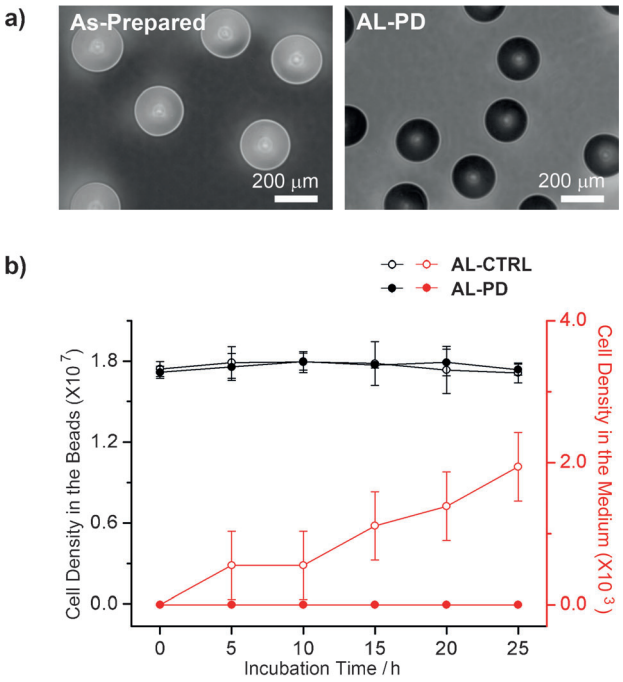

Figure 2. a) Phase-contrast microscopy images of the as-prepared and AL-PD beads after 1 hour incubation in the SD/-Trp/ + $CaCl_2$ medium. b) Cell densities (cells mL^{-1}) in the beads and in the medium after incubation in a low-nutrient medium at 5 °C.

the formation of AL-PD beads. As a control, the same coating procedures were performed without dopamine (AL-CTRL beads). The beads not containing *S. cerevisiae* cells were used for characterization. The Raman spectrum of the opaque AL-PD beads showed the characteristic bands of PD at about 1370 and 1580 cm^{-1}, corresponding to the stretching and deformation of the catechol moiety, respectively (Figure S1 a, b in the Supporting Information).[17] The scanning electron microscopy (SEM) images showed that the surface of the AL-PD beads comprised corrugated structures whereas the AL-CTRL beads had cracks as a result of dehydration (Figure S1 c). The thickness of the PD shell was estimated to be about 40 nm based on the transmission electron microscopy (TEM) image of microtomed AL-PD beads (Figure S1 d).

PD is a cytocompatible coating material and has been applied to the interfacing of living cells without significant loss of cell viability.[18] Additionally, we and others have previously shown that the PD layer was mechanically durable and permselective when individual cells were coated with PD.[19] To determine the cytocompatibility of the PD coating in the current system, we obtained the colony-forming unit (CFU) for the three yeast-laden bead samples: the as-prepared beads and the AL-PD and AL-CTRL beads. After bead degradation with citric acid (50 mM), serially diluted cell suspensions were spread on a synthetically defined tryptophan dropout (SD/-Trp) plate, and the cells were incubated at 30 °C for 48 hours. The log(CFU mL^{-1}) values were calculated to be 6.67 (for the as-prepared beads), 6.64 (for the AL-CTRL beads), and 6.61 (for the AL-PD beads), respectively. The fluorescein diacetate (FDA) and resazurin assays also confirmed that the PD-coating process was highly cytocompatible (Table S1).

The mechanical stability of the PD shell was tested using a swelling experiment. Ionotropic alginate beads generally swell when immersed in a monovalent ion-containing solution including cell culture medium, because the calcium cations in the alginate beads are replaced readily with monovalent cations and eluted into the solution.[20] Accordingly, we observed the swelling of the as-prepared beads after 1 hour incubation in the SD/-Trp medium supplemented with $CaCl_2$ (10 mM; SD/-Trp/ + $CaCl_2$), and the bead diameter increased from 192.2 μm to 232.5 μm (approximately 1.8-fold volume increase; Figure 2 a). In contrast, the AL-PD beads retained their sizes under the same conditions, indicating the physical toughness of the PD layer. The resistance of the AL-PD beads to swelling was further confirmed by investigating cell leakage. Although the alginate beads typically have a pore-size distribution of 5–200 nm,[21] the swelling causes onset and continual leakage of microbes by diffusion. For example, about 57 % cell leakage was detected in the case of Ca^{2+}-alginate beads encapsulating *S. cerevisiae* or *Oenococcus oeni* after immersion for 24 hours immersion in a low-nutrient broth at 10 °C.[22] We suspended the cell-laden alginate beads (AL-CTRL or AL-PD beads) in a low-nutrient medium at 5 °C and measured the cell densities both in the beads and in the medium at 5 hour intervals for one day. The hemocytometric measurements showed that the cell density (cell numbers per mL) in both beads was about 1.8×10^7 and did not change with incubation times up to 25 hours, indicating that the cells did not grow under the conditions (Figure 2 b, black lines). On the other hand, the yeast cells leaked from the AL-CTRL beads, and the cell density in the medium increased gradually with time (Figure 2 b, red lines). For example, after 5 hours incubation the cell density in the medium was 5.6×10^2 cells mL^{-1} and after 25 hours incubation it was 1.9×10^3 cells mL^{-1}. In stark contrast, we did not detect any cell leakage from the AL-PD beads under the same conditions.

Not only did the PD shell prevent swelling and cell leakage but it also protected the cells inside from harmful external stresses, such as enzymatic attack and UV-C irradiation. We used alginate lyase as a model for the enzymatic attack, because its digestion of alginate would lead to uncontrolled exposure and release of the cells to the outside, which could be investigated with ease by hemocytometric measurements.[23] The AL-CTRL or AL-PD bead suspension was immersed in a Tris-HCl buffer solution of alginate lyase (0.2 mg mL^{-1}), and the cell density in the buffer solution was measured at 0.5 hour intervals (Figure 3a). For

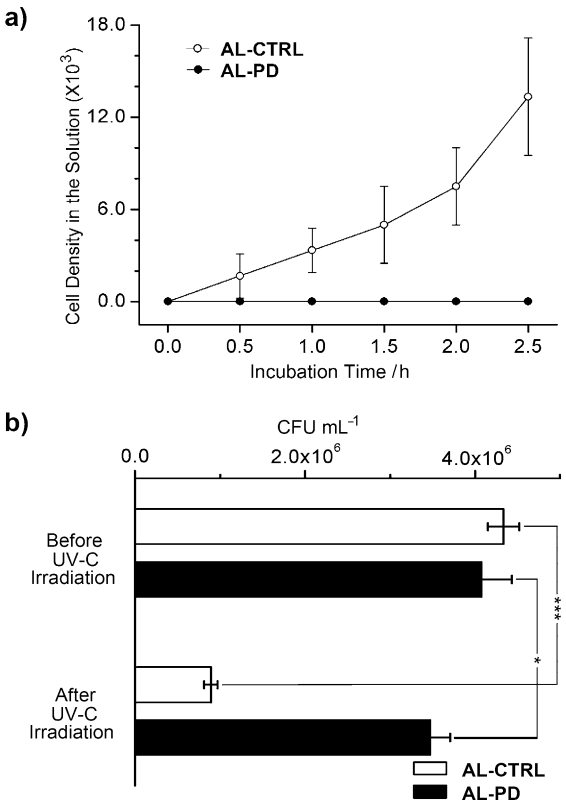

Figure 3. a) Cell densities (cells mL^{-1}) in the medium after the enzymatic digestion of alginate beads. b) Colony-forming units (CFU) of the encapsulated cells after 10 minutes irradiation with UV-C light. Statistical significance was analyzed by Student's *t*-test (*$p < 0.1$, ***$p < 0.001$).

the AL-CTRL beads, the cell density increased gradually with incubation time and reached 1.3×10^4 cells mL^{-1} after 2.5 hours, indicating alginate degradation and cell leakage. In contrast, we did not detect any cells in the buffer for the AL-PD beads. The results indicated that the PD shell precluded the penetration of the large enzymes, confirming the important characteristic of immunoprotection for the encapsulated cells.

UV light has deleterious effects on living organisms, causing mutagenic and cytotoxic DNA lesions.[24] UV-C light (wavelength: $\lambda = 100$–280 nm) is the most germicidal in the UV range and most microorganisms are killed even by a short exposure to UV-C radiation. Therefore, the protection of microbes against UV-C would be a clear-cut example of the cytoprotective capability of the PD shells.[25] After 10 minutes irradiation of the beads with light ($\lambda = 254$ nm; light power = 4 W), only approximately 20% of the cells in the AL-CTRL beads formed the colonies, but more than 85% of the cells survived the irradiation for the AL-PD beads (Figure 3b). In combination, these results indicate that the simple, one-step PD coating of the alginate beads encapsulating *S. cerevisiae* cells led to the formation of microcapsules that were mechanically tough and cytoprotective, and that prevented swelling.

The microbial growth was also suppressed by the PD shell. When incubated in a normal nutrient medium at 30°C, *S. cerevisiae* cells in the AL-CTRL beads grew continuously and leaked easily to the outside. The leaked yeast cells were observed in the medium after at least 5 hours of incubation in the confocal laser-scanning microscopy (CLSM) images (Figure 4). In contrast, the cell growth was suppressed greatly

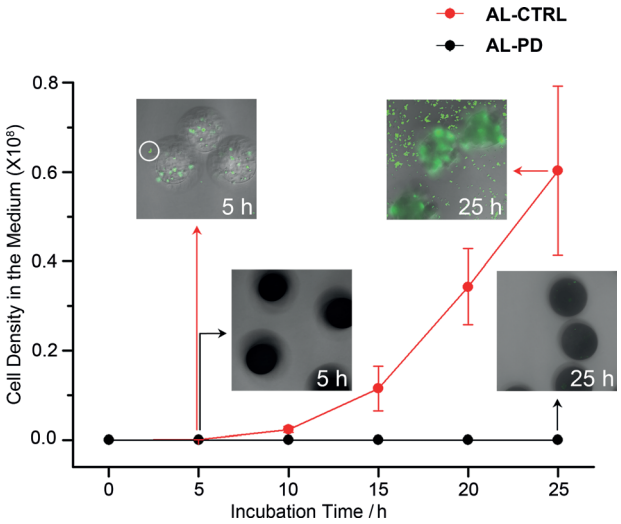

Figure 4. Cell densities (cells mL^{-1}) with increasing incubation time after incubation in the SD/-Trp/ + $CaCl_2$ medium. CLSM images were taken with focus on the cells in the medium. The white circle indicates the yeast cells in the medium.

in the AL-PD beads, and any leaked cells were not detected after 25 hours of incubation. The cell density in the AL-PD beads was measured to be 2.8×10^8 cells mL^{-1} after 25 hours culture, whereas that of AL-CTRL beads was 2.6×10^9 cells mL^{-1}, indicating that cell growth was suppressed by the PD coating (initial density: 1.8×10^7 cells mL^{-1}). The cells in the AL-PD beads were, however, metabolically active, indicated by the green fluorescence emitted from the cells.

In summary, we formed a multifunctional polydopamine (PD) coat on microbe-laden alginate microbeads. The alginate/PD core/shell capsules were mechanically durable and cytoprotective. Both microbe leakage from the microbeads and vulnerability of the cells to external attacks and stresses have been major bottlenecks to the practical use of microbial microencapsulation techniques in the areas where the spatiotemporal shielding of microbes against the outside is strictly required. We believe that this work provides a simple but versatile chemical tool to effectively isolate

and protect microbes from the outside as well as preventing their leakage. This substrate-independent cytoprotective coating method could also be applied to any type and size of capsule which encapsulates living cells, such as pancreatic islets, and may be employed in areas such as cellular medicine and biosensors.[26]

Experimental Section

Encapsulation of GFPuv-Yeast Cells: GFPuv-yeast cells were suspended in an SD/-Trp liquid medium and cultured in a shaking incubator at 30 °C for 18 hours. Sodium alginate was dissolved to a final concentration of 2.0 % (w/v) in a 10 mM Tris-HCl buffer solution (pH 8.5). The $CaCl_2$ solution (100 mM) was prepared by dissolving $CaCl_2$ in a Tris-HCl buffer solution (10 mM; pH 8.5). The cells were suspended in the sodium alginate solution (cell density: 1.8×10^7 cells mL^{-1}). For microencapsulation of GFPuv-yeast cells, the cell-containing suspension (1 mL) was jetted into the $CaCl_2$ solution (35 mL) with a 26-gauge needle. The flow rate was set to be 4 mL h^{-1} by a syringe pump with an applied voltage of 8.5 kV. The cell-laden microbeads were allowed to be hardened in the solution for 30 minutes, leading to the formation of the cell-laden as-prepared beads. The solution of the cell-laden as-prepared beads was shaken well and divided into two solutions of equal volume. The dopamine hydrochloride was added to one of the divided samples with a final concentration of 5 mg mL^{-1}, and the mixture was shaken at room temperature for 12 hours. After collecting by gravity sedimentation, the cell-laden AL-PD beads were washed five times with Tris-HCl buffer solution (10 mM; pH 8.5) of $CaCl_2$ (100 mM). As a control, the cell-laden AL-CTRL beads were generated with the other divided sample by the same procedures without dopamine hydrochloride.

Received: August 22, 2014
Revised: September 27, 2014
Published online: October 29, 2014

Keywords: cytoprotection · encapsulation · gels · nanostructures · polydopamine

[1] a) M. A. Fischbach, J. A. Bluestone, W. A. Lim, *Sci. Transl. Med.* **2013**, *5*, 179ps7; b) T. M. S. Chang, *Nat. Rev. Drug Discovery* **2005**, *4*, 221 – 235; c) S. Prakash, T. M. S. Chang, *Nat. Med.* **1996**, *2*, 883 – 887.
[2] M. T. Cook, G. Tzortzis, D. Charalampopoulos, V. V. Khutoryanskiy, *J. Controlled Release* **2012**, *162*, 56 – 67.
[3] A. Léonard, P. Dandoy, E. Danloy, G. Leroux, C. F. Meunier, J. C. Rooke, B.-L. Su, *Chem. Soc. Rev.* **2011**, *40*, 860 – 885.
[4] D. A. Stenger, G. W. Gross, E. W. Keefer, K. M. Shaffer, J. D. Andreadis, W. Ma, J. J. Pancrazio, *Trends Biotechnol.* **2001**, *19*, 304 – 309.
[5] a) A. Camilli, B. L. Bassler, *Science* **2006**, *311*, 1113 – 1116; b) M. Weitz, A. Mückl, K. Kapsner, R. Berg, A. Meyer, F. C. Simmel, *J. Am. Chem. Soc.* **2014**, *136*, 72 – 75.
[6] a) D. Velasco, E. Tumarkin, E. Kumacheva, *Small* **2012**, *8*, 1633 – 1642; b) E. Tumarkin, E. Kumacheva, *Chem. Soc. Rev.* **2009**, *38*, 2161 – 2168.
[7] F. Lim, A. M. Sun, *Science* **1980**, *210*, 908 – 910.
[8] a) V. Kozlovskaya, O. Zavgorodnya, Y. Chen, K. Ellis, H. M. Tse, W. Cui, J. A. Thompson, E. Kharlampieva, *Adv. Funct. Mater.* **2012**, *22*, 3389 – 3398; b) J. T. Wilson, W. Cui, V. Kozlovskaya, E. Kharlampieva, D. Pan, Z. Qu, V. R. Krishnamurthy, J. Mets, V. Kumar, J. Wen, Y. Song, V. V. Tsukruk, E. L. Chaikof, *J. Am. Chem. Soc.* **2011**, *133*, 7054 – 7064; c) J. T. Wilson, V. R. Krishnamurthy, W. Cui, Z. Qu, E. L. Chaikof, *J. Am. Chem. Soc.* **2009**, *131*, 18228 – 18229.
[9] C. R. Correia, R. L. Reis, J. F. Mano, *Biomacromolecules* **2013**, *14*, 743 – 751.
[10] M. Ma, A. Chiu, G. Sahay, J. C. Doloff, N. Dholakia, R. Thakrar, J. Cohen, A. Vegas, D. Chen, K. M. Bratlie, T. Dang, R. L. York, J. Hollister-Lock, G. C. Weir, D. G. Anderson, *Adv. Healthcare Mater.* **2013**, *2*, 667 – 672.
[11] a) J. H. Park, S. H. Yang, J. Lee, E. H. Ko, D. Hong, I. S. Choi, *Adv. Mater.* **2014**, *26*, 2001 – 2010; b) I. Drachuk, M. K. Gupta, V. V. Tsukruk, *Adv. Funct. Mater.* **2013**, *23*, 4437 – 4453; c) S. H. Yang, D. Hong, J. Lee, E. H. Ko, I. S. Choi, *Small* **2013**, *9*, 178 – 186; d) R. F. Fakhrullin, Y. M. Lvov, *ACS Nano* **2012**, *6*, 4557 – 4564.
[12] F. Godia, C. Casas, S. Sola, *Biotechnol. Prog.* **1991**, *7*, 468 – 470.
[13] W. S. Cheow, K. Hadinoto, *Biomacromolecules* **2013**, *14*, 3214 – 3222.
[14] Y. Zhou, E. Martins, A. Groboillot, C. Champagne, R. Neufeld, *J. Appl. Microbiol.* **1998**, *84*, 342 – 348.
[15] E. I. Rabea, M. E. Badawy, C. V. Stevens, G. Smagghe, W. Steubaut, *Biomacromolecules* **2003**, *4*, 1457 – 1465.
[16] H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426 – 430.
[17] B. Fei, B. Qian, Z. Yang, R. Wang, W. C. Liu, C. L. Mak, J. H. Xin, *Carbon* **2008**, *46*, 1795 – 1797.
[18] a) K. Kang, S. Lee, R. Kim, I. S. Choi, Y. Nam, *Angew. Chem. Int. Ed.* **2012**, *51*, 13101 – 13104; *Angew. Chem.* **2012**, *124*, 13278 – 13281; b) K. Kang, I. S. Choi, Y. Nam, *Biomaterials* **2011**, *32*, 6374 – 6380.
[19] a) B. Wang, G. Wang, B. Zhao, J. Chen, X. Zhang, R. Tang, *Chem. Sci.* **2014**, *5*, 3463 – 3468; b) S. H. Yang, S. M. Kang, K.-B. Lee, T. D. Chung, H. Lee, I. S. Choi, *J. Am. Chem. Soc.* **2011**, *133*, 2759 – 2797.
[20] X. Wang, H. G. Spencer, *Polymer* **1998**, *39*, 2759 – 2764.
[21] O. Smidsrød, G. Skjåk-Bræk, *Trends Biotechnol.* **1990**, *8*, 71 – 78.
[22] E. Callone, R. Campostrini, G. Carturan, A. Cavazza, R. Guzzon, *J. Mater. Chem.* **2008**, *18*, 4839 – 4848.
[23] H. Onoe, T. Okitsu, A. Itou, M. Kato-Negishi, R. Gojo, D. Kiriya, K. Sato, S. Miura, S. Iwanaga, K. Kuribayashi-Shigetomi, Y. T. Matsunaga, Y. Shimoyama, S. Takeuchi, *Nat. Mater.* **2013**, *12*, 584 – 590.
[24] R. P. Sinha, D.-P. Häder, *Photochem. Photobiol. Sci.* **2002**, *1*, 225 – 236.
[25] J. H. Park, K. Kim, J. Lee, J. Y. Choi, D. Hong, S. H. Yang, F. Caruso, Y. Lee, I. S. Choi, *Angew. Chem. Int. Ed.* **2014**, DOI: 10.1002/anie.201405905; *Angew. Chem.* **2014**, DOI: 10.1002/ange.201405905.
[26] D. Hong, M. Park, S. H. Yang, J. Lee, Y.-G. Kim, I. S. Choi, *Trends Biotechnol.* **2013**, *31*, 442 – 447.

