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Nanoimmobilization of Marine Epoxide Hydrolase of *Mugil cephalus* for Repetitive Enantioselective Resolution of Racemic Styrene Oxide in Aqueous Buffer

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We developed two nanoimmobilized biocatalyst systems of thermally unstable *Mugil cephalus* epoxide hydrolase (McEH) for enantioselective resolution of racemic styrene oxide in aqueous buffer. The recombinant and purified McEH enzyme was immobilized onto magnetic nanoparticles (Mag-NPs) via a two step process of enzyme precipitation and crosslinking. McEH enzyme was also adsorbed, precipitated, and cross-linked in/on polyaniline nanofibers (PANFs). The residual relative activity of free McEH, defined as the ratio of residual activity to the initial activity, was 8% after incubation at 30 °C for 80 h while those of McEH immobilized onto Mag-NPs and in/on PANFs were 15% and 33% in the same condition, respectively. McEH immobilizations onto Mag-NPs and in/on PANFs could be reused in seven repetitive batch reactions for enantioselective hydrolysis of racemic styrene oxide to prepare (*S*)-styrene oxide with 98% enantiomeric excess (*ee*) while retaining greater than 40–50% of their initial activity.

Keywords: Magnetic Nanoparticle, Polyaniline Nanofiber, Nanoimmobilization, Epoxide Hydrolase, Enantioselective Resolution.

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1. INTRODUCTION

Drugs with chiral centers are being developed as single enantiomers.[1] Various synthetic intermediates have been extensively supplied for the synthesis of chiral pharmaceuticals, of which chiral epoxides are one of the most useful intermediates.[2–5] Chiral epoxides can be prepared by chemical methods. Jacobsen's asymmetric epoxidation and hydrolytic kinetic resolution technology provide a commercial process to produce chiral epoxides with 98% enantiomeric excess (*ee*) enantiopurity in the presence of 1 mol% catalyst.[6, 7] For these chemical methods, however, heavy metal-based catalysts and organic solvents were employed.

Currently, much effort has focused on applying green chemistry methods to develop a process that minimizes the use and generation of hazardous substances.[8] Biocatalysis is a green chemistry approach that offers several advantages such as mild reaction conditions, high selectivity, low formation of undesired by-products, and catalysis in aqueous systems.[9] The use of enzymes for the production of chiral compounds is one of the most important research areas in green chemistry. Biocatalytic preparation of chiral epoxides by epoxide hydrolase (EH) is considered as an emerging alternative to chemical methods.[10–12] EH catalyzes the enantioselective hydrolysis of racemic epoxides to prepare chiral epoxides.[13]

Commercial implementation of biocatalysis is often impeded by high costs and instability of enzymes. The immobilization of enzymes using nanomaterials is proposed as a potential solution.[14–15] Magnetic nanoparticles (Mag-NPs) have been used for various biotechnology applications,[16–27] and the immobilization of enzymes onto Mag-NPs in the form of enzyme coatings has been shown to enhance enzyme stability and reusability.[28–29] Glucose oxidase enzyme was successfully immobilized in and on polyaniline nanofibers (PANFs) in a three step process of enzyme adsorption, precipitation and cross-linking

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(EAPC), resulting in good enzyme stability as well as high enzyme loading.[30]

Recently, a novel recombinant EH biocatalyst was developed by protein engineering of a EH from the marine fish, *Mugil cephalus*.[31] A triple-point mutant consisting of F193Y for spatial orientation of the nucleophile, W200L for preventing electron density overlap, and E378D for good charge relay in the active site was developed by comparative modeling-inspired site-directed mutagenesis.[32] The engineered *M. cepahlus* EH (McEH), however, is unstable. In the present work, we evaluated nano-immobilization methods based on different nano-structured supports such as Mag-NPs and PANFs (Fig. 1) and analyzed the stability characteristics of immobilized McEH. The reusability of immobilized McEH was also investigated for repetitive preparation of enantiopure (*S*)-styrene oxide from racemic substrate in an aqueous buffer.

2. MATERIALS AND METHODS

2.1. Materials

Sodium phosphate monobasic, sodium phosphate dibasic, glutaraldehyde, ammonium sulfate, Tris, ammonium persulfate, aniline, racemic styrene oxide and (*S*)-styrene oxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Culture medium components were purchased from Merck and Difco.

2.2. Cell Culture Conditions and McEH Recombinant Protein Expression

Recombinant *E. coli* BL21 (DE3) harboring the recombinant pET-21b(+)/McEH plasmid containing *M. cephalus* EH gene was cultured at 37 °C and 180 rpm. Expression of McEH protein was induced at 15 °C by adding 1 mM IPTG to the cell culture and was then maintained for 24 h. Cells were harvested at specific time points, and the specific activity of McEH was analyzed by chiral gas chromatography (GC).

2.3. Recombinant Protein Purification

The cells were harvested by centrifugation and resuspended in 50 mM sodium phosphate lysis buffer containing 300 mM NaCl, 10 mM imidazole, 10% (v/v) glycerol, and 0.5% (v/v) Triton X-100. DNase, lysozyme, and phenylmethanesulfonyl fluoride were added to final

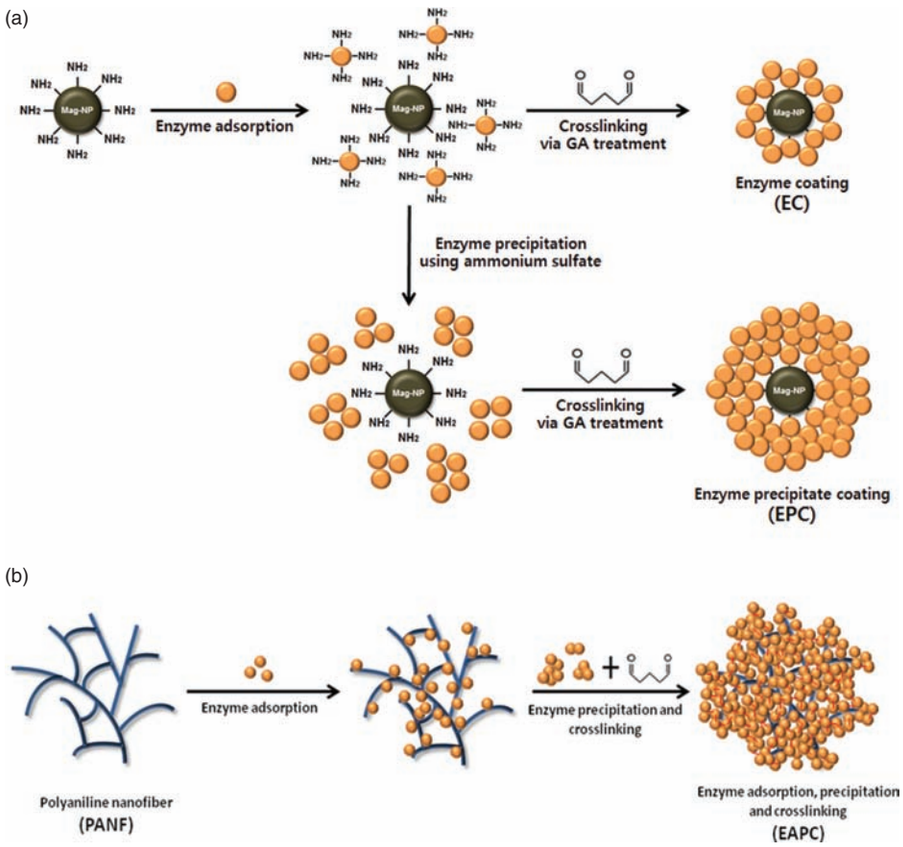

Fig. 1. Schematic representations of nanoimmobilization of *M. cephalus* epoxide hydrolase (McEH). (a) Enzyme precipitate coating (EPC) onto magnetic nanoparticles (Mag-NPs) and (b) enzyme adsorption, precipitation and cross-linking (EAPC) in/on polyaniline nanofibers (PANFs).

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concentrations of 0.02 mg/mL, 1 mg/mL, and 1 mM, respectively. The suspension solution was homogenized on ice with a Vibra Cell VCX 400 ultrasonic processor for 20 min. The cell debris was centrifuged down (13,000 rpm, 20 min) at 4 °C, and the supernatant was filtered using a 0.2 μm membrane to obtain a clear extract. The resulting supernatant containing His-tagged McEH and chaperones was loaded onto a Ni-Sepharose column equilibrated with 50 mM phosphate buffer (pH 8.0), 0.5 M KCl, and 5 mM imidazole. The column was washed with 30 mL of the same buffer containing 10 mM imidazole at a flow rate of 5 mL/min to remove chaperones. Finally, His-tagged McEH was eluted with the same buffer containing 250 mM imidazole. The active fraction was desalted using a HiTrap™ desalting column. The expressed gene products were analyzed by 12% (v/v) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously.[32]

2.4. Preparation of Immobilized McEH

2.4.1. Enzyme Precipitate Coating (EPC) of McEH on Magnetic Nanoparticles (Mag-NPs)

Iron oxide nanoparticles with average size of 10 nm were synthesized and further functionalized to graft amino groups on them as previously reported.[33] Amino group functionalized Mag-NPs were mixed with enzyme solution for 2 h. To proceed with the EPC immobilization technique, the enzyme precipitation was performed in the ammonium sulfate solution (50% w/v) for 30 min. Glutaraldehyde treatment (GA) was then performed at a final GA concentration of 0.1% (v/v) at 4 °C under tilt-shaking for overnight. After washing with 10 mM phosphate buffer (pH 7.0), unreacted aldehyde groups of GA were blocked in 100 mM Tris buffer (pH 7.4), and the samples were excessively washed by using 10 mM phosphate buffer (pH 7.0). The EPC sample of McEH on Mag-NPs (Fig. 1(a)) was stored at 4 °C until use.[28, 29]

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2.4.2. Enzyme Adsorption, Precipitation and Crosslinking (EAPC) of McEH in and on Polyaniline Nanofibers (PANFs)

PANFs were prepared by a rapid mixing of monomer and initiator as previously reported.[30, 34] In details, monomer (aniline) and initiator (ammonium persulfate) were dissolved separately in 1 M HCl, and then immediately mixed and shaken at 200 rpm at room temperature for overnight. The sample of PANFs was excessively washed with distilled water, and dried under ambient condition before use.

McEH was immobilized in and on PANFs via a technique of enzyme adsorption, precipitation, and crosslinking as described before.[30] In details, the McEH solution was mixed with PANFs, and the mixture was shaken at 150 rpm for 1 h. After this adsorption step, the enzyme precipitation was performed in the ammonium sulfate solution (50% w/v). Finally, the enzyme crosslinking was performed at the GA concentration of 0.5% (v/v) under tilt-shaking at 4 °C for overnight. The capping and washing processes was performed in the same way as for the EPC immobilization onto Mag-NPs. The final sample of EAPC with McEH in/on PANFs (Fig. 1(b)) was stored at 4 °C until use.[30]

2.5. Enzyme Activity and Stability Measurements

The measurement of enzyme activity was conducted in 1 mL 100 mM KH_2PO_4 buffer (pH 7.4) containing the enzyme sample. Enantioselective hydrolysis was initiated by the addition of 10 mM racemic styrene oxide, and the mixture was shaken at 230 rpm in an incubator at 30 °C. The reaction was stopped by the extraction using an equal volume of cyclohexane.

The rate of enantioselective hydrolysis and enantiomeric excess ($ee = 100 \times ([S]-[R])/([S]+[R])$, where $[S]$ and $[R]$ represent the concentration of enantiomers, respectively) were analyzed using a GC equipped with a flame ionization detector (FID) and BETA DEX column (30 m $\times$ 0.25 mm $\times$ 0.25 μm). The temperatures of oven, detector and injector were 100, 220 and 220 °C, respectively. The carrier gas was nitrogen in a flow rate of 0.5 mL/min. The stabilities of free and immobilized McEH were analyzed by measuring the enzyme activity time-dependently. The relative activity was calculated from the ratio of residual activity at each time point to the initial activity of each sample. The enzyme samples were incubated at 4, 15, and 30 °C, respectively.

2.6. Recycled Uses of Immobilization Enzyme

After each batch of enzyme reaction, the immobilized McEH onto Mag-NPs or in/on PANFs was separated by using a magnet or centrifugation at 13000 rpm for 10 min, respectively. The separated immobilized enzymes were washed twice with the reaction buffer and then added to a new reaction solution for repeated batch reactions of enantioselective hydrolysis.

3. RESULTS AND DISCUSSION

3.1. Production and Purification of Recombinant McEH

Recombinant marine EH from *M. cephalus* (McEH) possessed high activity and a broad substrate spectrum with high enantioselectivity for kinetic resolution of racemic epoxide substrates. However, its stability is low even at moderate reaction temperatures such as 30 °C. In order to enhance the stability of McEH and its reusability in a repetitive batch mode, we immobilized McEH using nanomaterials such as Mag-NPs and PANFs. In the present work, the immobilization of McEH onto Mag-NPs or in/on

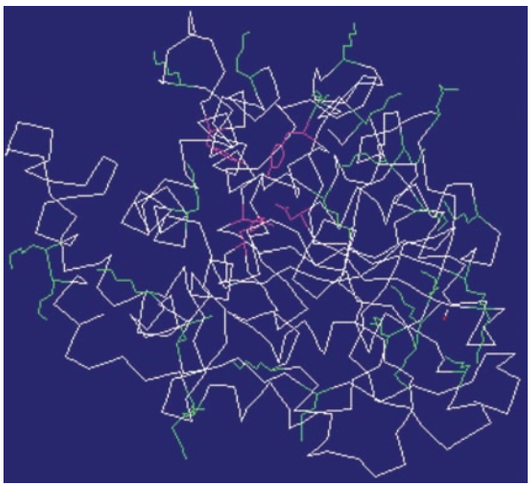

Fig. 2. Homology model of McEH protein. The red indicates active site and green represents the location of lysine residues.

PANFs employed glutaraldehyde cross-linking between amine groups of McEH. The number of lysines, possessing amine functional groups, in a McEH protein was 32, representing the second most abundant amino acid in McEH. The locations of lysine groups in McEH were identified by homology-modeling and predicted to be outside the catalytic active site (Fig. 2). Therefore, we expect that cross-linking between McEH and glutaraldehyde would not cause a significant loss of catalytic activity.

In order to develop McEH biocatalysts immobilized onto Mag-NPs and in/on PANFs, McEH enzyme was prepared. Recombinant McEH was produced in *E. coli* in the presence of chaperones with IPTG induction at exponential growth phase. The recombinant protein was purified on Ni-Sepharose columns. The expression level and purification of McEH protein were analyzed by SDS-PAGE (Fig. 3). Purified McEH appeared as a single band on SDS-PAGE with a molecular mass of approximately 45 kDa.

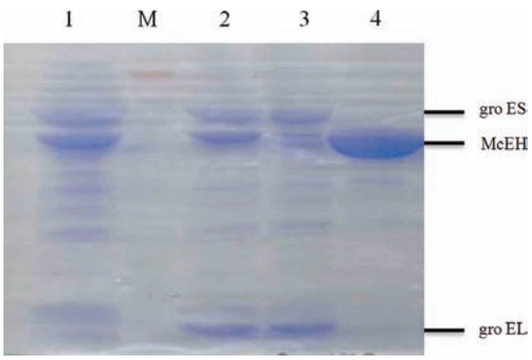

Fig. 3. SDS-PAGE of the purified recombinant McEH protein expressed in the presence of chaperons (pGro7). Lane 1: whole cells expressing recombinant McEH proteins, *M*: molecular marker, lane 2: lysate of the recombinant cells, lane 3: unbinding protein mixture, lane 4: the purified recombinant McEH proteins.

3.2. Stability of Immobilized McEH Onto Mag-NPs and in/on PANFs

McEH was immobilized onto Mag-NPs via an approach of enzyme precipitate coatings (EPCs), which consists of two steps: enzyme precipitation and crosslinking (Fig. 1(a)). On the other hand, McEH was immobilized in/on PANFs in a three step approach of enzyme adsorption, precipitation and crosslinking (Fig. 1(b)). In order to compare the stability of free and immobilized McEH, their residual activity at each time point was measured, and the relative activity, defined to be the ratio of residual activity to the initial activity, was used for the stability comparison of free and immobilized enzymes. First, the stability of free enzyme was checked at 4, 15, and 30 °C. Free McEH enzyme maintained about 60% of relative activity after 220 h incubation at 4 and 15 °C. On the other hand, the activity of free enzyme rapidly decreased at 30 °C, showing a negligible enzyme activity after 120 h of incubation (Fig. 4).

The stability of McEH immobilized onto Mag-NPs and in/on PANFs at 4, 15 and 30 °C was also investigated. For McEH immobilized onto Mag-NPs, the relative activity were 86 and 63% after 220 h of incubation at 4 and 15 °C, respectively. For McEH immobilized in/on PANFs, 68 and 67% of the initial activity remained after 220 h incubation at 4 °C and 15 °C, respectively. The stability of both immobilized McEH samples showed reasonably good stability at 4 °C and 15 °C.

The relative activity of McEH immobilized onto Mag-NPs decreased to 21 and 5% of the initial activity after 40 and 220 h of incubation at 30 °C, respectively (Fig. 5). For McEH immobilized PANFs, 17% of the initial activity was retained after 220 h at 30 °C. PANF-based immobilization of McEH was more stable than Mag-NP-based

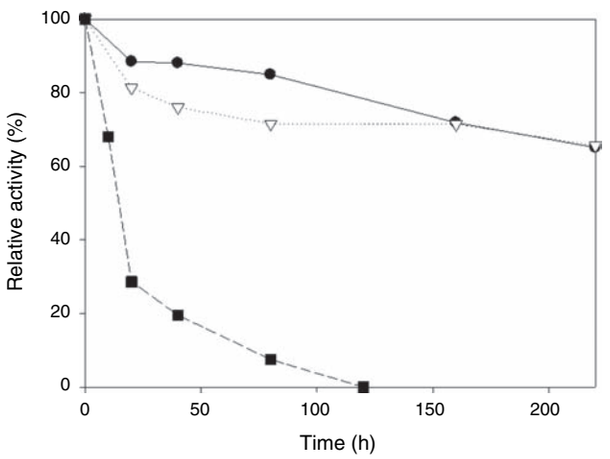

Fig. 4. Thermal deactivation of the free McEH enzyme in aqueous buffer solution (100 mM potassium phosphate, pH 7.5) at each temperature (4, 15 and 30 °C). The relative activity was determined from the ratio of the residual activity at each sampling time to the initial activity. Symbol: •, 4 °C; ▽, 15 °C; ■, 30 °C.

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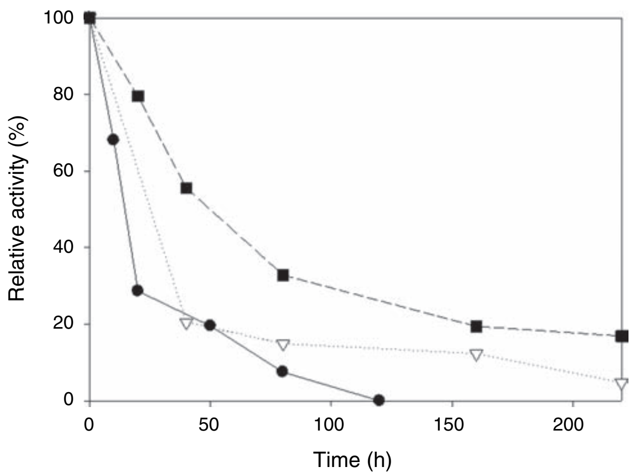

Fig. 5. Prolonged catalytic activity of the nano-immobilized McEH by EPC onto Mag-NPs and EAPC in/on PANFs. The relative activity was determined from the ratio of the residual activity at each sampling time to the initial activity. Symbol: ●, free enzyme; ▽, EPC-based immobilization onto Mag-NPs; ■, EAPC-based immobilization in/on PANFs.

immobilization because PANFs provide a porous structure that would prevent the enzymes from denaturation via confinement effect. Although the relative activities of both types of immobilized McEH was low after 220 h incubation at 30 °C, the stability of immobilized McEH was better than that of free McEH showing a negligible activity in the same condition. After incubation at 30 °C for 80 h, the residual relative activity of free McEH was 8% while those of McEH immobilized onto Mag-NPs and in/on PANFs were 15% and 33% in the same condition, respectively (Fig. 5). The immobilized enzymes were more stable than free enzymes at various temperatures because of effective enzyme precipitation and crosslinking that inhibit the enzyme denaturation and leaching. Site-directed mutagenesis of McEH using comparative modeling enhanced its enantioselective hydrolysis activity by 35-fold; thus, only a few minutes were required to reach 98% *ee*.[20] Therefore, 80 h is sufficient for McEH-catalyzed enantioselective hydrolysis of racemic styrene oxide in aqueous buffer.

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3.3. Recycling of Immobilized McEH in Repetitive Batch Mode

Since Mag-NPs have superparamagnetic properties, McEH immobilized onto Mag-NPs was separated for recycled uses by using a magnet. McEH immobilized PANFs could also be separated upon centrifugation. To evaluate the reusability of nanoimmobilized McEHs, the residual catalytic activity was measured whenever immobilized McEHs were reused. As the number of reuses increased, the residual activity gradually decreased (Table I). McEH immobilized onto Mag-NPs or in/on PANFs was reused in seven repetitive batch reactions for enantioselective hydrolysis of racemic styrene oxide for preparing 98% *ee* (S)-styrene oxide in aqueous buffer. The residual activity

Table I. Recycling of McEH immobilized onto magnetic nanoparticles and in/on polyaniline nanofibers. The relative activity was calculated by the ratio of residual activity to the initial activity after repetitive reactions. EPC-Mag-NPs and EAPC-PANFs represent magnetic nanoparticle-based and polyaniline nanofiber-based immobilizations, respectively.

	Relative residual activity (%)	
Number of enzyme recycling	EPC-Mag-NPs	EAPC-PANFs
1st	100	100
2nd	91.1	100
3rd	81.1	74.5
4th	70.8	72.8
5th	46.1	67.5
6th	41.9	58.8
7th	39.1	52.8

of McEH immobilized onto Mag-NPs or in/on PANFs was above 50% of the initial activity after four and seven repetitions, respectively. This result indicates that nanoimmobilization of McEH onto magnetic nanoparticles or in/on polyaniline nanofibers enhances both enzyme stability and reusability in the enantioselective hydrolysis of racemic styrene oxide in aqueous buffer.

4. CONCLUSIONS

Recombinant McEH was immobilized onto Mag-NPs and in/on PANFs using the EPC and EAPC methods, respectively. The resulting EPC and EAPC of McEH showed enhanced enzyme stability at various temperatures and under recycled uses due to effective prevention of enzyme denaturation and leaching. Stabilized McEH in the form of nanoimmobilization using Mag-NPs and PANFs would provide a potential use of McEH in the enantioselective hydrolysis of racemic styrene oxide in aqueous buffer. The present success of McEH stabilization can be employed for further improvements of other EH enzymes together with various enzymes for their successes in applications where the poor enzyme stability hampers their practical uses.

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References and Notes

1. S. C. Stinson, *Chem. Eng. News* 72, 38 **(1994)**.
2. M. Breuer, K. Ditrich, T. Habicher, and B. Hauer, *Angew. Chem. Int. Edit.* 43, 788 (**2004**).
3. J. W. Lee, S. S. Yoo, S. H. Park, H. S. Kim, and E. Y. Lee, *Biotechnol. Bioprocess. Eng.* 8, 306 (**2003**).

4. W. J. Choi and C. Y. Choi, *Biotechnol. Bioprocess Eng.* 10, 167 **(2005)**.
5. S. H. Hwang, C. Y. Choi, and E. Y. Lee, *J. Ind. Eng. Chem.* 16, 1 **(2010)**.
6. M. Tokunaga, J. F. Larrow, F. Kakiuchi, and E. N. Jacobsen, *Science* 277, 936 **(1997)**.
7. T. Yoon and E. N. Jacobsen, *Science* 299, 1691 **(2003)**.
8. R. A. Sheldon, *Chem. Soc. Rev.* 41, 1437 **(2012)**.
9. N. Ran, L. Zhao, Z. Chen, and J. Tao, *Green Chem.* 10, 361 **(2008)**.
10. E. Y. Lee and M. L. Shuler, *Biotechnol. Bioeng.* 98, 318 **(2007)**.
11. S. H. Hwang, C. Y. Choi, and E. Y. Lee, *Biotechnol. Bioprocess Eng.* 13, 453 **(2008)**.
12. A. Steinreiber and K. Faber, *Curr. Opin. Biotechnol.* 12, 552 **(2001)**.
13. A. Archelas and R. Furstoss, *Curr. Opin. Chem. Biol.* 5, 112 **(2001)**.
14. J. Kim, J. W. Grate, and P. Wang, *Chem. Eng. Sci.* 61, 1017 **(2006)**.
15. J. Kim, J. W. Grate, and P. Wang, *Trends Biotechnol.* 26, 639 **(2008)**.
16. S. H. Choi, H. B. Na, Y. I. Park, K. An, S. G. Kwon, Y. Jang, M. Park, J. Moon, J. S. Son, I. C. Song, W. K. Moon, and T. Hyeon, *J. Am. Chem. Soc.* 130, 15573 **(2008)**.
17. H. B. Na, I. C. Song, and T. Hyeon, *Adv. Mater.* 21, 2133 **(2009)**.
18. F. Wang, C. Ma, X. Zeng, C. Li, Y. Deng, and N. He, *J. Biomed. Nanotechnol.* 8, 786 **(2012)**.
19. L. He, Z. Li, J. Fu, F. Wang, C. Ma, Y. Deng, Z. Shi, H. Wang, and N. He, *J. Nanosci. Nanotechnol.* 11, 2256 **(2011)**.
20. S. H. Choi, H. S. Kim, I. S. Lee, and E. Y. Lee, *Biotechnol. Lett.* 32, 1685 **(2010)**.
21. K. S. Lee, M. H. Woo, H. S. Kim, E. Y. Lee, and I. S. Lee, *Chem. Commun.* 25, 3780 **(2009)**.
22. R. Singh and H. S. Nalwa, *J. Biomed. Nanotechnol.* 7, 489 **(2011)**.
23. H. -X. Ren, L. Liu, C. Liu, S. -Y. He, J. Huang, J. -L. Li, Y. Zhang, X. -J. Huang, and N. Gu, *J. Biomed. Nanotechnol.* 7, 677 **(2011)**.
24. D. Šponarová, D. Horák, M. Trchová, P. Jendelová, V. Herynek, N. Mitina, A. Zaichenko, R. Stoika, P. Lesný, and E. Syková, *J. Biomed. Nanotechnol.* 7, 384 **(2011)**.
25. A. Arkhis, A. Elaissari, T. Delair, B. Verrier, and B. Mandrand, *J. Biomed. Nanotechnol.* 6, 28 **(2010)**.
26. M. Wang, J. Zhang, Z. Yuan, W. Yang, Q. Wu, and H. Gu, *J. Biomed. Nanotechnol.* 8, 624 **(2012)**.
27. C. Ma, C. Li, N. He, F. Wang, N. Ma, L. Zhang, Z. Lu, Z. Ali, Z. Xi, X. Li, G. Liang, H. Liu, Y. Deng, L. Xu, and Z. Wang, *J. Biomed. Nanotechnol.* 8, 1000 **(2012)**.
28. J. Lee, Y. Lee, J. K. Youn, H. B. Na, T. Yu, H. Kim, S. -M. Lee, Y.-M. Koo, J. H. Kwak, H. G. Park, H. N. Chang, M. Hwang, J.-G. Park, J. Kim, and T. Hyeon, *Small* 4, 143 **(2008)**.
29. B. Lee, D. Lopez-Ferrer, B. C. Kim, H. B. Na, Y. I. Park, K. K. Weitz, M. G. Warner, T. Hyeon, S. W. Lee, R. D. Smith, and J. Kim, *Proteomics* 11, 309 **(2011)**.
30. H. Kim, I. Lee, Y. Kwon, B. C. Kim, S. Ha, J. H. Lee, and J. Kim, *Biosens. Bioelectron.* 26, 3908 **(2011)**.
31. S. J. Lee, H. S. Kim, S. J. Kim, S. Park, B. J. Kim, M. L. Shuler, and E. Y. Lee, *Biotechnol. Lett.* 29, 237 **(2007)**.
32. S. H. Choi, H. S. Kim, and E. Y. Lee, *Biotechnol. Lett.* 31, 1617 **(2009)**.
33. J. Lee, D. Lee, E. Oh, J. Kim, Y.-P. Kim, S. Jin, H.-S. Kim, Y. Hwang, J. H. Kwak, J.-G. Park, C.-H. Shin, J. Kim, and T. Hyeon, *Angew. Chem. Int. Edit.* 117, 7593 **(2005)**.
34. J. Huang and R. B. Kaner, *Angew. Chem. Int. Ed.* 43, 5817 **(2004)**.

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