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Poly(ethylene glycol) Conjugated Enzyme with Enhanced Hydrophobic Compatibility for Self-Cleaning Coatings

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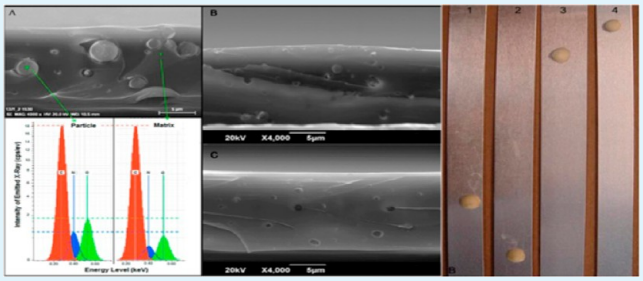
ABSTRACT: Enzyme-based smart materials constitute a rapidly growing group of functional materials. Often the natively evolved enzymes are not compatible with hydrophobic synthetic materials, thus significantly limiting the performance of enzymes. This work investigates the use of a polyethylene glycol (PEG)-conjugated detergent enzyme for self-cleaning coatings. As a result, PEG conjugated α-amylase demonstrated a much more homogeneous distribution in polyurethane coatings than the parent native enzyme as detected by both fluorescent microscopy and scanning electron microscopy (SEM) equipped with energy-dispersive X-ray spectroscopy (SEM-EDX). Additionally, the conjugated enzyme showed enhanced retention in the coating and much improved thermal stability with a halflife of 20 days detected at 80 °C and over 350 days under room temperature. Such coating-incorporated enzyme afforded interesting self-cleaning functionality against starch-based stains as examined through a slipping drop test.



KEYWORDS: *PEG conjugation, compatibility, enzyme distribution, enzyme retention, self-cleaning coating, smart materials*

INTRODUCTION

Coatings play important roles in protecting the solid substrates from contamination, corrosion, and wear. Recently, there has been a growing interest in developing smart coatings with novel functionalities including self-cleaning, self-healing, self-reporting, antimicrobes, and detoxification.[1] For the purpose of protection against stain contamination, one traditional way is to manipulate the surface physical properties of the coatings, including application of super hydrophobic materials, thus preventing potential binding of sticky hydrophilic stains.[2−6] For example, Ebert et al. recently reported that spraying hydrophobic silica particles onto micropattened epoxy surfaces could produce highly hydrophobic coatings with a water contact angle of 168°, much higher than the 80° observed for the original epoxy material.[7] As alternative to such nonadhesive coatings, coatings that contain a small amount of chemically active agents that can catalyze *in situ* partial degradation of adhesive components of stains promise a new type of functional coatings. We may classify herein such chemically active coatings as self-cleaning coatings. The use of small amounts of active catalysts for self-cleaning eliminates restriction requirements for surface properties of the coatings and, therefore, can be prepared with broader selections of coating materials. One widely examined chemical agent for this application is TiO_2.[8−10] TiO_2 chemically breaks down the stains when exposed to sunlight, following a photocatalytic reaction mechanism. As a recent example, Ganesh et al. reported the preparation of self-cleaning coatings (tested with a dye, Alizarin Red) with TiO_2 electrospun with polyvinyl acetate/*N*,*N*-dimethyl acetamide on glass surfaces.[11]

As alternatives to inorganic catalysts that largely lack selectivity (for example, they may not be able to distinguish stains versus hosting organic coating materials), bioactive enzymes may be considered as smart catalysts for self-cleaning as they can target sticky components of stains with high selectivities. So far little has been reported on the use of bioactive enzymes for self-cleaning coatings. Nevertheless, people have demonstrated the feasibility of developing a variety of functional coatings with enzymes. Eby et al. reported preparation of antimicrobial coatings for medical instruments that applied lysozyme along with silver nanoparticles,[12] while several research groups have reported antibacterial coatings prepared with antimicrobial peptides (AMPs, also highly selective).[13−15] Tasso et al. reported an antifouling coating for underwater structures with enzymes.[16] Russell et al. demonstrated the preparation of polyurethane coatings for detoxification against nerve agents and pesticides with cross-linked fluorophosphatase.[17,18]

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One critical challenge in preparing bioactive coatings is the compatibility between enzyme molecules and coating materials. Toward that, previous research has demonstrated that conjugation of enzymes with synthetic polymers could significantly affect the hydrophobicity/hydrophilicity of enzymes.[19−22] Inspired by that, this work investigates the feasibility and efficacy of using polymer-conjugated enzymes for preparation of enzyme-based self-cleaning coatings. We expect that, by efficiently integrating digestive enzymes such as those that have been widely applied for detergent formulas, we can prepare smart self-cleaning coatings that selectively attack *in situ* sticky components of stains upon contact. Specifically we investigate the effect of PEG conjugation on the performance of a detergent enzyme, α-amylase, for polyurethane self-cleaning coatings.

EXPERIMENTAL CONDITIONS AND METHODS

Materials. Coating materials including polyacrylic resin (Desmophen A870) and polyisocyanate (Desmodur N3600) were provided by Bayer Corp. (Pittsburgh, PA). Surfactant BYK-333 was obtained from BYK-Chemie (Wallingford, CT). α-Amylase powder from *Bacillus subtilis* (EC 3.2.1.1) was purchased from Amano Enzyme Inc. (Nagoya, Japan). PEG *N*-hydroxysuccinimide (NHS) ester (MW 1000) was obtained from NANOCS (New York, NY). All other chemicals and solvents of chemical agent grade were purchased from Sigma Chemical Co. (St. Louis, MO).

PEG Conjugation of Enzyme. Native α-amylase enzyme was purified by using ultrafiltration with regenerated celluloses membrane (MW cutoff of 30 kDa purchased from Millipore, Billerica, MA) before being applied in this study. The ultrafiltration was conducted with a 50 mL cell, with each load of 2 g of enzyme purified with 3 refills of 50 mM pH 7.5 PBS buffer solution. Enzyme solution (diluted to a concentration of 140 mg protein/mL, as determined by Bradford assay) of 1 mL was first added to a 5-mL glass vial and incubated in an ice bath with magnetic stirring. PEG conjugation was initialized by adding a desired amount (depending on modification ratio) of PEG-NHS that was predissolved in DMSO. After 4 h of reaction, ultrafiltration centrifugation was conducted to remove free PEG modifier (five rounds of purification with fresh buffer of the same volume of the original sample for each round) with a 50 KDa MW cutoff membrane (Pall Corporation, Ann Arbor, MI). The protein content in the final purified product was determined via Bradford assay, and the total amount of recovered product showed a typical yield of 84% (based on the total amount of added enzyme). Enzyme concentration was then adjusted back to 140 mg/mL for coating preparation or diluted further for SDS-PAGE and the activity assay.

Coating Preparation. PEG conjugated enzyme solution (600 μL) was mixed with 2.1 g of Desmodur A870 in a 20-mL glass vial, 500 μL of *n*-butyl acetate and 100 μL of BYK-333 (17% in butyl acetate) were then added. The mixture was emulsified mechanically (with an IKA RW 11 Lab-egg stirrer) for 1 min at 1500 rpm, subsequently transferred to a second glass vial containing 0.8 g of Desmophen N3600, and then mixed for 1 min at 1500 rpm. The resulting mixture was coated onto aluminum panels or foils for preparation of coating samples for performance evaluation or SEM analysis by using a draw-down method (predetermined thickness, 20 μm) with an 8-path wet film applicator (Paul N. Gardner, FL). The prepared coatings were placed in a hood cabinet for 10 min to evaporate the butyl acetate and then cured at 70 °C for 4 h.

Coating Characterization. The enzyme-functionalized coatings were analyzed by using an inverted fluorescence microscope (Olympus IX70) with blue light (450−490 nm) excitation. The cross-section of bioactive coating was inspected with SEM (JSM-5800 JEOL Co., Tokyo, Japan), which was coupled with a Bruker AXS XFlash 4010 Silicon Drift Detector EDX (Bruker). The cross-section samples were prepared by tearing coatings hosted on aluminum foil sheets. The sample was sputtered with Au−Pd and mounted on a thin specimen split mount (Ted Pella Inc., Redding, CA). All data was collected under the same conditions as specified in the following: working distance 12 mm; takeoff angle 35°; accelerating voltage 20 kV, which was estimated to be more than 2.5 times of the escape energy for any element peaks (C, N, O); magnification 4000×; count rate adjusted between 1.8 and 2.5 kcps; duration of 15 s for stable peak acquisition. The peak and background values of C, N, O elemental analyses were quantified using the PB-ZAF standardless method of the Quantax program package with iterative corrections, by using fundamental factors to correct for the effects of atomic number. Normalized element concentrations based on corrected peak heights were used for the ratio calculation.

Self-Cleaning Functionality Test. Mayonnaise Subway sandwich sauce was used to examine the self-cleaning functionality against real-life stains through a slipping drop test. Sample coatings (including regular PU reference coating) were prepared on alumina panels. Each coating panel was loaded with one stain drop (0.2 g) of sandwich sauce at one end of the panel and was then set vertical while starting timing the slipping of the stain drop toward the other end within the premarked distance. The repeated self-cleaning functionality tests were conducted after the coatings were rinsed for 10 s with fresh deionized water and blotted-dry with Kimtech wipes.

Coating and Enzyme Activity Assay. The activities of the native α-amylase, PEG conjugated α-amylase, and coated enzyme was measured by using a colorimetric method with 3,5-dinitrosalicylic acid as the chromogen.[23] The substrate solution contained 1% (w/v) soluble potato starch in 20 mM sodium phosphate pH 6.9 buffer. Coated panels were cut into smaller pieces of 1.5 cm × 1.6 cm before being tested, with 1 mL of substrate solution applied for one coating specimen, incubated under room temperature for 3 min. For native enzyme and PEG conjugated enzyme, 10 μL of enzyme sample solution was added into 1 mL of substrate solution and tested under the same conditions. At the end of the reaction, 1 mL of 96 mM 3,5-dinitrosalicylic acid was added to the reaction solution and incubated in boiling water for 15 min before being cooled down in an ice bath. The equivalent of reducing sugar was determined with a Cary 50-Varian UV−vis spectrometer at 540 nm. One unit of α-amylase activity is defined as the amount of enzyme that produces 1.0 mg of reducing sugar from starch in 3 min at room temperature. Thermo stability and enzyme retention were examined by measuring residual enzyme activities (through the above procedure) of concerned samples as a function of conditioning and reuse history.

RESULTS AND DISCUSSION

Detergent enzymes are digestive hydrolases normally applied in solution formulas to selectively degrade stain-forming adhesive biomolecules (mostly polysaccharides, lipids, and proteins), with surfactant help to remove reaction residues immediately from targeted surfaces. Because of the high selectivity, different enzymes may be needed in commercial formulas depending on the types of stains targeted. α-Amylase, a model detergent enzyme selected for this study, can efficiently break down long-chain carbohydrates especially amylose to small basic units such as maltose and maltotriose. The enzyme is highly soluble in water, one important feature that is preferred for detergent formulas but makes it difficult to disperse the enzyme in a solid phase hydrophobic polymer coating as required for the current work. Conjugation with PEG was therefore conducted for α-amylase in an attempt to improve its compatibility with polyurethane coatings. Linear PEG-NHS, which has been applied as an efficient modifier for enzymes and protein drugs through lysine residues,[24] was examined in the current study. Being a glycoprotein, α-amylase also possesses 19 lysine residuals exposed to its molecular outer surface,[25] providing points for hydrophobicity modification with surface sugar groups untouched for affinity retention toward carbohydrate substrates. Unreacted PEG modifier was removed via ultra-filtration to purify the conjugated enzyme. SDS polyacrylamide

gel electrophoresis tests showed that the modification was efficient with no native enzyme left (evident from lacking of unmodified enzyme in the sample) when the molar ratio between the modifier and enzyme was 10:1 (lane 1 of Figure 1,

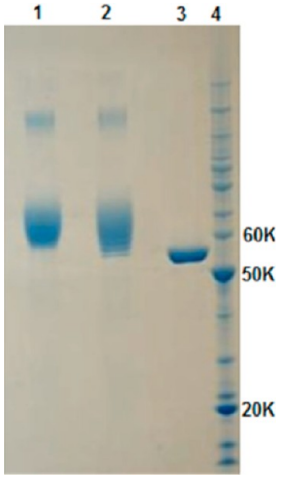


Figure 1. Electrophoresis (SDS-PAGE) analysis of PEG-conjugated α-amylase. lane 1, 10-fold PEG conjugation sample; lane 2, 5-fold PEG conjugation sample; lane 3, native α-amylase; lane 4, standard protein MW markers.

henceforward denoted as a 10-fold modification). The molecular weight of the modified enzyme fell in the range of 58−70 kDa for a 10-fold modification, indicating the number of PEG attachment is in the range of 5−15 (as the native enzyme showed a MW band of 53−55 kDa, Figure 1). Compared to that, the 5-fold modification (molar ratio between the modifier and enzyme was 5:1) led to modified enzyme of MW of 55−70 kDa (no. of PEG attachment, 2−15) and left a small portion of enzyme unmodified. The number of PEG attachment is quite reasonable considering the number of surface lysine groups of α-amylase, and the broad range of number of attachment indicated heterogeneity of the modification reaction, a result of the random PEG attachment.[26]

The PEG-conjugated enzyme demonstrated good retention of enzyme activity. Compared to native enzyme, PEG-conjugated α-amylase was less active (20% loss at maximum) at low temperatures, yet much more active for temperatures above 60 °C (Figure 2). For that we believe it is a result of the combined effects of activity loss due to the modification and stability enhancement of the conjugated enzyme, similar to other chemically modified enzymes.[27,28] The stability enhancement is evident in that the modified enzyme did not show much activity loss as the temperature increased from 50 to 70 °C (Figure 2), while the native enzyme lost 70% activity.

The conjugation with PEG apparently improved the compatibility of the enzyme with polyurethane as reflected through the protein distribution in thin film coatings. The enzyme-containing coatings were prepared through a standard draw-down method with the enzyme as one additive to the standard coating formula. The total protein content in the finished coating was controlled to be around 3% (w/w). The distribution of α-amylase was investigated by using inverted fluorescence microscopy. For coatings prepared with the native enzyme, bright green aggregates were found with diameters in the order of several micrometers, while the PEG-conjugated enzyme generated much more uniform distribution (Figure

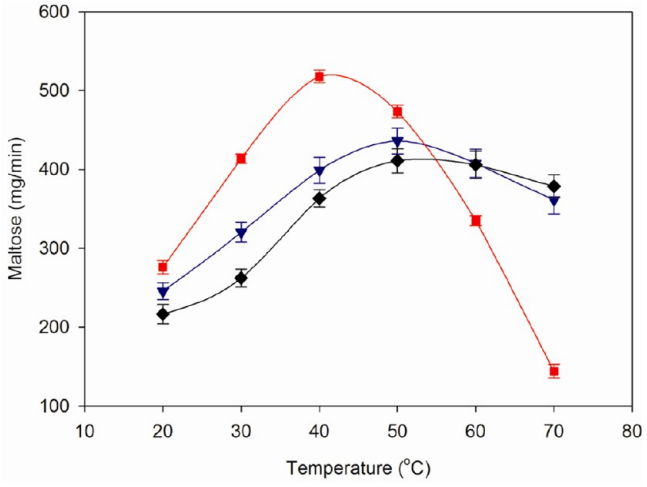


Figure 2. Effect of PEG conjugation on the activity of α-amylase: ■, native α-amylase; ▼, 5-fold PEG conjugated α-amylase; ♦, 10-fold PEG conjugated α-amylase.

3A). SEM analysis of the top surface also indicated enzyme aggregates exposed to the outer surface (Figure 3B) and again showed finer aggregates for PEG-conjugated enzyme.

The coatings were further examined with SEM-EDX analysis for verification of enzyme distribution. This is a method frequently used for elemental characterization of solid samples.[29] By measuring the energy of characteristic X-ray emission from atoms at the spotted locations of the sample, EDX along with SEM affords microscale chemical distribution analysis. Results verified that the fine particulates formed in the coatings were protein aggregates (Figure 4). As SEM-EDX analysis showed (Figure 4A), the ratio of C/N of the particulates was 3.5 ± 0.4, similar to that of native α-amylase but much lower than that of the bulk phase of the polyurethane coating (7.7 ± 0.1). The degree of modification also made a difference in enzyme distribution (Figure 4B,C). While enzyme aggregates with a diameter on the order of 1 μm were formed for conjugated enzyme with 5-fold modification, only submicrometer particulates could be detected for samples of 10-fold modification. The hydrophobicity of the coating did not show significant changes, with the water contacting angle slightly reduced from 80° for reference standard polyurethane coating to 75° and 78° for coatings prepared with enzyme of 5- and 10-fold modification, respectively. This may be a good reflection of changes in surface topology, with enzyme aggregates covered only a very small portion of the surface area of the coatings (see Figure 3B). Addition of PEG led to no apparent change in the coating's hydrophobicity, while coatings prepared with native enzyme showed a contact angle of 73°.

The formation of particulates allows part of the aggregates exposed to the outer surface, thereby providing surface bioactivity. That also allows the enzyme to be washed away with fluidic samples. Our activity tests of the coatings with aqueous solutions of starch showed that PEG conjugation could help to retain the enzyme from being washed away. Activity tests showed that freshly prepare coatings have specific activities ranged from 1200, 70, and 37 mU/cm^2 for native, 5-fold, and 10-fold PEG modification of α-amylase, respectively. The high specific activity of the native α-amylase coating may have been a result of the larger enzyme aggregates (Figure 3), and the quick release of enzyme to the reaction solution (the released free enzyme can provide a much higher activity than

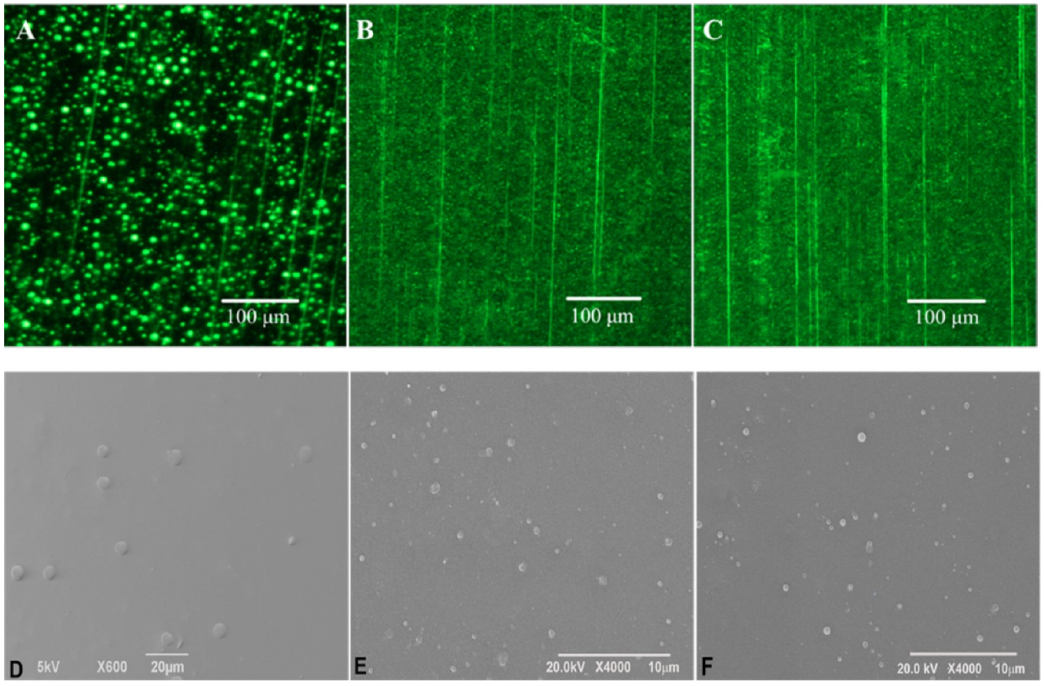


Figure 3. Effect of PEG conjugation on enzyme distribution in polyurethane coating. (Top) Fluorescence microscopy images of native α-amylase coating (A), 5-fold PEG conjugated α-amylase coating (B), and 10-fold PEG conjugated α-amylase coating (C). (Bottom) SEM images of native α-amylase coating (D), 5-fold PEG conjugated α-amylase coating (E), and 10-fold PEG conjugated α-amylase coating (F).

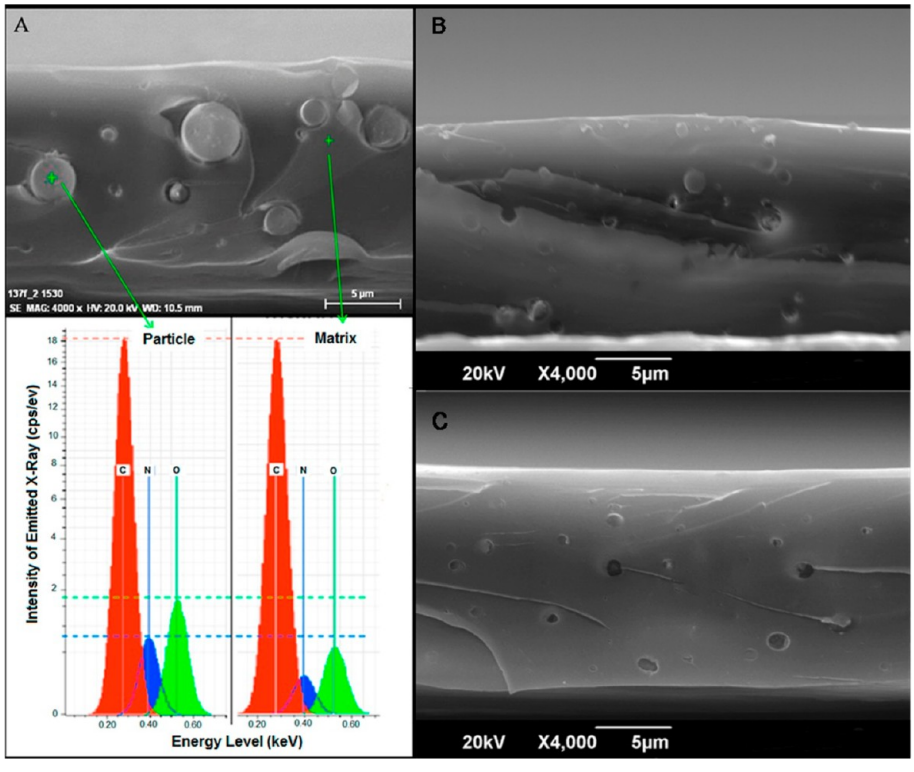


Figure 4. Enzyme distribution in polyurethane coating via SEM-EDX analysis. SEM images of cross-section area of (A) native α-amylase coating, along with elemental analysis of particular matters (bottom), which indicated comparison in composition between the bulk phase polymer and particulates; (B) 5-fold PEG conjugated α-amylase coating; (C) 10-fold PEG conjugated α-amylase coating.

surface-attached). Enzyme release is evident for native α-amylase from the observation that the coatings quickly lost its activity, with no activity detected after 4 cycles of repeated activity tests (as shown in Figure 5). To further verify the enzyme release, we also immersed the coating samples in water (with a sample size of 1.5 cm × 1.6 cm, each in 1 mL of DI water) for 30 min and found that about 8% of the total enzyme loading was released into the water, which gave an activity that accounted to be 90% of the original coating activity. Compared to that, the coatings of 5-fold modification enzyme showed

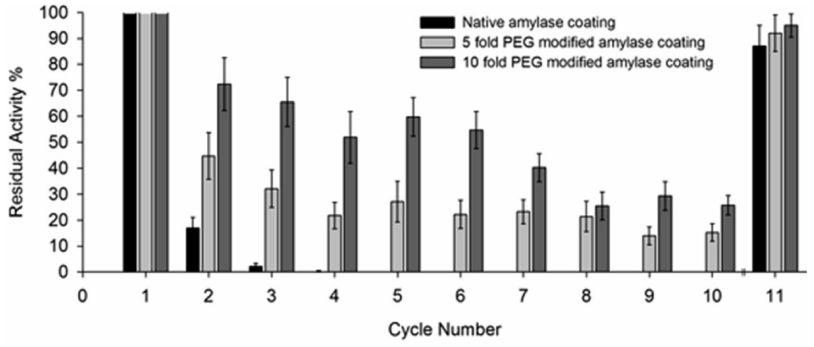


Figure 5. Effect of PEG conjugation on enzyme retention in polyurethane coating. (Activities of coatings were examined repeatedly after each test cycle; data for 11th test cycle were obtained by wiping the coating surface with tissue paper after the 10th test cycle).

Scheme 1. PEG Conjugation Enhances Enzyme Retention via Molecular Entanglement

activity loss in the first 3 test cycles but maintained activity almost unchanged thereafter. Enzyme retention was even better for coatings with 10-fold modification enzyme throughout the 10 test cycles. After 10 test cycles, both coatings with PEG conjugated enzyme showed relatively stable activities in the order of 11 U/cm^2. We assume here that PEG side-chains, which are compatible with polyurethane, may have been embedded in the polyurethane phase and got entangled with the polymer matrix, thereby tethering the enzyme to the coating (Scheme 1).

For a consideration of coating applications, the enzyme embedded beneath the surface of coating may become functional should stain matters diffuse through cavities, fractures, or wear damages. Additionally, the enzyme aggregates entrapped inside the coatings may serve as a reserved supply to amend enzyme loss. One test showed that very gentle wiping of the coating without any apparent physical damage effectively exposed enzyme molecules initially hidden in action, with the coating activities found almost fully recovered by wiping the surface with tissue papers after 10 reuse cycles (data for 11th reuse cycle of Figure 5). We believe that since the enzyme aggregates close to the top surface are mostly partially embedded in the coating, with some aggregates may be barely exposed, the wiping may help the aggregates that are mostly covered to be better exposed.

Thermostability is one of the most concerning properties for biomolecule-based materials in practical applications. Thermostability of PEG conjugated α-amylase coatings were investigated by heating the coating at 80 °C. The residual bioactivity of the coating was measured as a function of heating time. As a result, the half lifetimes at 80 °C for coatings with native enzyme, 5-fold, and 10-fold modification enzyme were found to be 16, 22, and 25 days, respectively (Figure 6). Free native enzyme (dry powder) only showed a half-life as 3 days for the same test. Other tests also showed that the halflife of 5-fold modification coating was as long as 350 days under ambient conditions. The longer half lifetimes for PEG modified enzyme

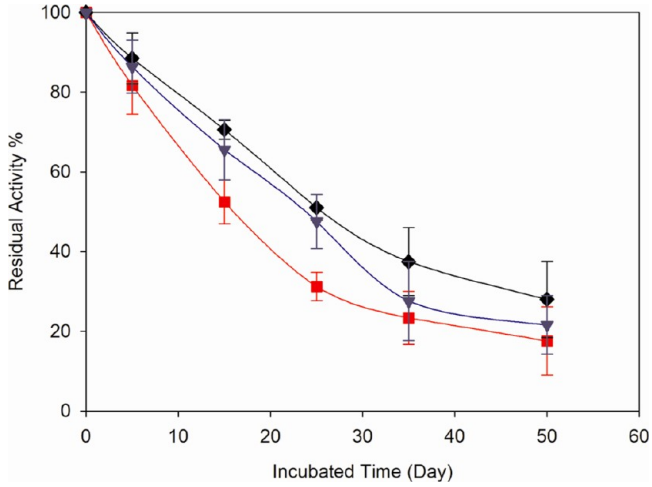
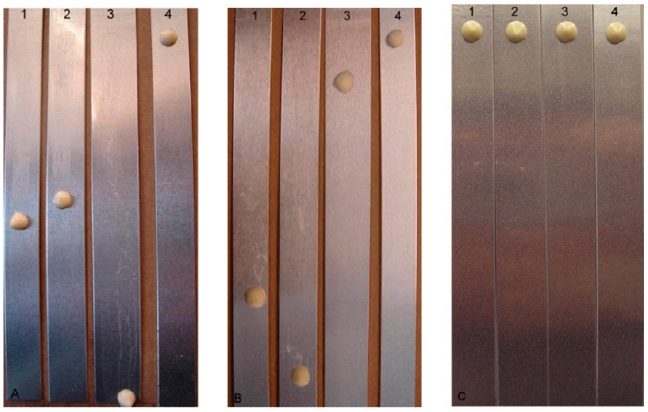


Figure 6. Thermostability of enzyme-based coating at 80 °C. ■, native α-amylase; ▼, 5-fold PEG conjugated α-amylase coating; ♦, 10-fold PEG conjugated α-amylase coating.

coatings may be attributed to the PEGylation-induced thermostability enhancement (see data shown in Figure 2). In addition, confining the enzyme within the polymeric network of the coating may also help the enzyme to stand against protein chain unfolding, a common mechanism of thermo inactivation of enzymes placed in a solution phase. This is similar to observations with other forms of immobilized enzymes including α-amylase.[30]

Self-cleaning functionality of such prepared coatings against real world stains was tested. Mayonnaise sandwich dressing that contains starch, vegetable oil, egg yolks, water, and salts was applied for this test. The coated alumina panels were tilted vertically after the stain was applied at one end of the panel, and the time needed for the stain drop to slide down to the bottom was recorded as an indication of the efficiency of self-cleaning (Figure 7). The refrence standard polyurethane coating did not



(A) (B) (C)

Figure 7. Self-cleaning functionality test of polyurethane coatings. (A) The first-time functionality test (pictures were taken at 1 min after the panels were tilted vertically, with panel 1, 5-fold PEG conjugated α-amylase coating; panel 2, 10-fold PEG conjugated α-amylase coating; panel 3, native α-amylase coating; panel 4, reference polyurethane coating without α-amylase. (B) The 4th reuse functionality test (pictures were taken at 15 min after the panels were tilted vertically, the same samples as tested in part A). (C) Slipping drop test on different reference coatings (panel 1, BSA coating; panel 2, regular coating with pure PEG-1K; panel 3, 5-fold PEG conjugated α-amylase coating after enzyme inactivation under 160 °C for 24 h; panel 4, 10-fold PEG conjugated α-amylase coating after enzyme inactivation under 160 °C for 24 h; pictures were taken at 15 min after the panels were tilted vertically).

promote any motion of the stain, while all other coatings containing enzyme showed the desired functionality (with the stains slided down). The stain drop on the native enzyme coating slipped to the bottom in less than 1 min (attributing to the activity of free enzyme released from the coating). In the case of PEG-conjugated enzyme, stains slid relatively slower and reached the bottom in 3 min (Figure 7A). The difference in slipping rate may be attributed to the apparent activity difference of the freshly prepared coatings (as discussed above regarding Figure 5). The coated panels were repeatedly tested after being rinsed with water, and after 3 reuses, the native enzyme coating showed barely visible functionality (with very short distance of slipping after 15 min, as shown in lane 3 in Figure 7B), while coatings with PEG conjugated enzyme maintained their cleaning functionality well. This observation agreed well with the results of coating surface activity tests (Figure 5). The coating panel of native enzyme was cut after 4 reuse cycles (no self-cleaning effect observed at that point) and tested for residue bioactivity. Interestingly, the coating still retained a small amount of enzyme with an activity of 8 mU/cm^2, which may indicate the minimun value of surface enzyme activity needed for self-cleaning functionality. No self-cleaning effect was observed for coatings with either an inert protein (BSA) or inactivated enzymes (by heating the coatings under 160 °C for 24 h) (Figure 7C), indicating the self-cleaning functionality is a result of the enzyme activity.

CONCLUSIONS

In summary, PEG conjugation improved the compatibility between enzyme and polyurethane, thus helping to disperse and retain the enzyme in thin film coating. The coated enzyme afforded highly selective surface bioactivity which allows the coating to selectively degrade stain-forming biomolecules, avoiding formation of stains. Polyurethane coatings prepared with PEG conjugated α-amylase demonstrated excellent self-cleaning against starch-rich stains such as sandwich sauces. Such self-cleaning coatings may help to reduce water consumption (by shortening the washing time) and environmental contamination (by eliminating the need of chemical detergents) for washing when applied to automobiles or household facilities. Combined with the enhanced thermo-stability, such enzyme-based coatings promise a variety of practical applications and, depending on the enzymes applied, may enable smart coatings offering functionalities including self-cleaning, self-reporting, and antibacteria.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Provder, T. *Smart Coatings II*; Baghdachi, J., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, 2009; p 3.
(2) Ganesh, V. A.; Raut, H. K.; Nair, A. S.; Ramakrishna, S. *J. Mater. Chem.* **2011**, *21*, 16304−16322.
(3) Ma, M.; Hill, R. M.; Lowery, J. L.; Fridrikh, S. V.; Rutledge, G. C. *Langmuir* **2005**, *21*, 5549−5554.
(4) Jiang, L.; Zhao, Y.; Zhai, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 4338−4341.
(5) Yang, Y. M.; Szu, P. S.; Lee, Y. L. *Nanotechnology* **2007**, *18*, 465604−465610.
(6) Li, Y.; Cai, W.; Duan, G. *Chem. Mater.* **2007**, *20*, 615−624.
(7) Ebert, D.; Bhushan, B. *J. Colloid Interface Sci.* **2012**, *368*, 584−591.
(8) Yu, J.; Zhao, X.; Zhao, Q.; Wang, G. *Mater. Chem. Phys.* **2001**, *68*, 253−259.
(9) Sato, O.; Zhang, X.; Taguchi, M.; Einaga, Y.; Murakami, T.; Fujishima, A. *Chem. Mater.* **2005**, *17*, 696−700.
(10) Yaghoubi, H.; Taghavinia, N.; Alamdari, E. K. *Surf. Coat. Technol.* **2010**, *204*, 1562−1568.
(11) Ganesh, V. A.; Nair, A. S.; Raut, H. K.; Walsh, T. M.; Ramakrishna, S. *RCS Adv.* **2012**, *2*, 2067−2072.
(12) Eby, D. M.; Luckarift, H. R.; Johnson, G. R. *ACS Appl. Mater. Interfaces* **2009**, *7*, 1553−1560.
(13) Kazemzadeh-Narbat, M.; Kindrachuk, J.; Duan, K.; Jenssen, H.; Hancock, R. E.; Wang, R. *Biomaterials* **2010**, 9519−9526.
(14) Pfeufer, N. Y.; Hofmann-Peiker, K.; Mühle, M.; Warnke, P. H.; Weigel, M. C.; Kleine, M. *J. Bone Joint Surg. Am.* **2011**, *93*, 840−846.
(15) Fulmer, P. A.; Lundin, J. G.; Wynne, J. H. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1266−1270.
(16) Tasso, M.; Pettitt, M. E.; Cordeiro, A. L.; Callow, M. E.; Callow, J. A.; Werner, C. *Biofouling* **2009**, *25*, 505−516.
(17) Drevon, G. F.; Russell, A. J. *Biomacromelcules* **2000**, *1*, 571−576.

(18) Drevon, G. F.; Danielmeier, K.; Federspiel, W.; Stolz, D. B.; Wicks, D. A.; Yu, P. C.; Russell, A. J. *Biotechnol. Bioeng.* **2002**, *79*, 785−794.
(19) Zhu, G. Y.; Wang, P. *J. Am. Chem. Soc.* **2004**, *126*, 11132−11133.
(20) Narayanan, R.; Zhu, G. Y.; Wang, P. *J. Biotechnol.* **2007**, *128*, 86−92.
(21) Virginia, D.; Kaar, J. L.; Russell, A. J.; Lele, B. S. *Biomacromolecules* **2008**, *9*, 1348−1351.
(22) Narayanan, R.; Stottrup, B. L.; Wang, P. *Langmuir* **2009**, *25*, 10660−10665.
(23) Yoo, Y. J.; Hong, J.; Hatch, R. T. *Biotechnol. Bioeng.* **1987**, *30*, 147−151.
(24) Hermanson, G. T. *Bioconjugate Techniques*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2008; p 171.
(25) Kagawa, M.; Fujimoto, Z.; Momma, M.; Takase, K.; Mizuno, H. *J. Bacteriol.* **2003**, *185*, 6981−6984.
(26) Soares, A. L.; Guimaraes, G.; Polakiewicz, M. B.; Pitombo, R. N.; Neto, J. A. *Int. J. Pharm.* **2002**, *237*, 163−170.
(27) Cruz, J. I.; Gonzalez, G.; Arana, A. *Bioconjugate Chem.* **2006**, *17*, 1093−1098.
(28) Martınez, J. A.; Sola, R. J.; Castillo, B.; Colon, H. R.; Rivera, I.; Barletta, G.; Griebenow, K. *Biotechnol. Bioeng.* **2008**, *101*, 1142−1149.
(29) Goldstein, J. I. *Scanning Electron Microscopy and X-Ray Microanalysis*, 3rd ed.; Newbury, D., Joy, D., Lyman, C., Echlin, E., Lifshin, E., Sawyer, L., Michael, J., Eds.; Kluwer Academic Publisher: New York, 2003; p 1.
(30) Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2003**, *42*, 3336−3337.