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Simultaneously and Separately Immobilizing Incompatible Dual-Enzymes on Polymer Substrate *via* Visible Light Induced Graft Polymerization

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Abstract

Developing facile and mild strategy to construct multi-enzymes immobilization system has attracted considerable attentions in recent years. Here a simple immobilization strategy called visible light induced graft polymerization that can simultaneously and separately encapsulate two kinds of enzymes on one polymer film was proposed. Two incompatible enzymes, trypsin and transglutaminase (TGase) were selected as model dual-enzymes system and simultaneously immobilized on two sides of low-density polyethylene (LDPE) film. After immobilization, it was found that more than 90% of the enzymes can be embedded into dual-enzymes loaded film without leakage. And the activities of both separately immobilized enzymes were higher than the activities of mixed co-immobilized enzymes or the sequential immobilized ones. This dual-enzymes loaded film (DEL film) showed excellent recyclability and can retain > 87% activities of both enzymes after 4 cycles of utilization. As an example, this DEL film was used to conjugate a prodrug of cytarabine with a target peptide. The successful preparation of expected product demonstrated that the separately immobilized two enzymes can worked well together to catalyze a two-step reaction.

1. Introduction

Immobilization of enzymes on or within a support is of great importance both scientifically and industrially [1-7]. Their moderate reaction conditions, high regio-

selectivity, simple operation and reusability have made them potential in many sustainable applications [8-12]. In previous researches, many works focused on single enzyme immobilization system [13, 14]. However, in many complex reactions, multi-enzymes working together are needed [15, 16]. It is still very challenging to efficiently immobilize multi-enzymes in an organized way on one substrate.

One convenient method to fix several enzymes on one substrate is called co-immobilization, which usually immobilizes the mixture of enzymes like a single enzyme [17-20]. For example, Liang et al. [21] mixed glucose oxidase (GOx) and horseradish peroxidase (HRP) together and encapsulated them into a hydrogel fiber made from Zn^{2+} and adenosine monophosphate. It was found that both of the immobilized enzymes retained more than 70% of their initial activities after 15 days storage, and they were even more stable against the external environment than free enzymes. Although it is very simple, co-immobilization is only applicable to the enzymes that do not interfere with each other. Therefore, it is significant to explore alternative immobilization strategy toward organizing multi-enzymes in different area of substrate to avoid mutual interference. Recently, a great number of efforts have been devoted to constructing separated immobilization systems [22-25]. Inspiring by mitochondria, Shi et al. [26] prepared hybrid double membrane microcapsules. One kind of enzyme was embedded inside the microcapsules, while the other kind of enzyme was fixed in the inter membrane space. Comparing to the mixing co-immobilization method, this separated immobilization method significantly improved the selectivity, activity and stability of enzymes. Zhang et al. [27] sequentially absorbed organophosphate hydrolase (OPH) and acetylcholine esterase (AChE) *via* layer-by-layer strategy on polyethyleneimine/DNA modified multi-walled carbon nanotubes. This dual-enzymes immobilization system was used to discriminate

between paraoxon and carbaryl pesticides, and it also exhibited good stability, sensitivity and reproducibility. Despite the obvious benefits of these contributions, the complicated process to localize each enzyme greatly hindered their practical application and the enzymes immobilized in the outer layers may block the activities of enzymes which immobilized in the inner layers. Moreover, some harsh conditions such as organic solvents, UV irradiation, acid or alkali may have to be used to realize the fabrication of the elaborately designed partition structure, but these factors may affect the activities of the immobilized enzymes [28, 29]. Last but not least, when the incompatible enzymes system was involved, the enzymes at the interface of different part could still affect each other [30]. Hence, it is still a significant and challenging task to develop simple and mild approach to separately immobilize multi-enzymes for practical application.

In this study, an extremely simple and mild technique, called visible light induced graft polymerization [31-33], was used to immobilize two enzymes on polymer substrate separately and simultaneously. The mechanism of this technique composed of two chemical steps (Scheme 1). Firstly, isopropyl thioxanthone (ITX) was coupled to both side of low-density polyethylene (LDPE) film under UV irradiation (Scheme 1a). Secondly, the formed ITX semipinacol (ITXSP) dormant groups were photolyzed under visible light to generate surface carbon radical. And then the surface radical could initiate graft crosslinking polymerization of poly(ethylene glycol) diacrylate (PEGDA) (**Scheme S1**). When the enzymes/PEGDA solutions were cast onto each side of LDPE-ITXSP and irradiated by visible light (Scheme 1b), photografting reaction occurred on two sides and two enzymes could be encapsulated into different PEG networks isolated by the LDPE film. With the help of a photomask, two micropatterns loaded with different enzymes grafted on LDPE could be easily

obtained. Compared with other separated immobilization methods, this strategy showed several advantages: 1) the immobilization of enzymes was performed under visible light and room temperature, which is mild enough to preserve the activity of enzymes; 2) the swelling of the PEG molecular network was markedly restricted by LDPE film thus is favorable to prevent the leakage of enzymes [31]; 3) it can immobilize two enzymes *via* a one-step reaction and greatly simplified the immobilization process; 4) two enzymes were completely separated by the film, eliminating the possibility of mutual interference.

Scheme 1 Schematic Route of (a) Planting the ITXSP on Both Side of LDPE Film under UV Irradiation; (b) Preparation of Patterned DEL Film *via* Visible Light-Induced Graft Polymerization

Herein, trypsin and transglutaminase (TGase) were chosen as model enzymes to fabricate the dual-enzymes loaded film (DEL film). TGase is one enzyme which can incorporate chemicals containing primary amines (e.g. lysine) into the protein

substrate which contains γ -glutaminy residues [34-35]. Trypsin is one specific digestive enzyme which can cut arginine or lysine on the carboxyl end of proteins or peptides [36-38]. When the two enzymes were mixed together, TGase may crosslink the trypsin and at the same time trypsin may hydrolyze TGase [39]. Therefore, completely separate immobilizing them on one substrate can not only retain their activities but also allow them to catalyze multi-steps reaction effectively.

To the best of our knowledge, this is the first time to provide a simple method that can immobilize two enzymes on one support separately and simultaneously. And we believe that this strategy is also applicable to immobilize other biomacromolecules.

2. Experimental

2.1. Grafting ITXSP on LDPE

Firstly, ITX solution (acetone as the solvent, 0.1 mL, 5 mmol mL⁻¹) was dropped onto a quartz plate, and then a 4 cm $\times$ 4 cm LDPE film was placed onto the quartz plate to spread out the solution uniformly. Secondly, ITX solution (acetone as the solvent, 0.1 mL, 5 mmol mL⁻¹) was dropped onto the front side of the LDPE film and then another quartz plate was placed onto the film to spread out the solution uniformly. The LDPE film with ITX solutions on both sides was eventually placed between the two quartz plates. Thirdly, this system was irradiated under high-pressure mercury lamp (wavelength 254 nm, 9 mW cm⁻²) for 5 min at room temperature to obtain LDPE-ITXSP film. After the irradiation reaction, LDPE-ITXSP film was washed with excess acetone to remove the residual ITX. Finally, this film was dried in a vacuum oven at room temperature.

2.2. Preparation of DEL Film

TGase (0.15 mg), Tris-HCl (100 mM, 37.5 μ L, pH=7.0) and PEGDA (M_w =575, 22.5 μ L) were mixed together to form the Solution A. Trypsin (0.43 mg), Tris-HCl (100 mM, 37.5 μ L, pH=7.0) and PEGDA (M_w =575, 22.5 μ L) were mixed together to form the Solution B. Firstly, Solution A (60 μ L) was cast onto quartz plate A and then a LDPE-ITXSP film was placed onto this quartz plate to spread out the solution uniformly. Secondly, Solution B (60 μ L) was dropped onto the front side of the LDPE-ITXSP film and then quartz plate B was placed onto the film to spread out the solution uniformly. Finally, this system was placed under the xenon lamp (filter was added with band pass of 380-700 nm, irradiation intensity was 3 mW cm⁻² at 420 nm) for 30 min at room temperature. After the grafting reaction, the DEL film was washed with Tris-HCl buffer five times to remove the unimmobilized enzymes. To prepare the patterned DEL film, the only difference was that a photomask was used to replace the quartz plate B.

To prepare the TGase or trypsin loaded film, Solution A or B (60 μ L) was cast onto a LDPE-ITXSP film and then the system was sandwiched between two quartz plates. This system was placed under the same xenon lamp for 30 min at room temperature. After the grafting reaction, the TGase or trypsin loaded film was washed with the same buffer for five times to remove the unimmobilized enzymes.

To prepare the Control A film, Solution A and B (60 μ L for each solution) were mixed uniformly to form the Solution C. Firstly, Solution C (60 μ L) was cast onto quartz plate A and then a LDPE-ITXSP film was placed onto this quartz plate to spread out the solution uniformly. Secondly, the rest of the Solution C (60 μ L) was dropped onto the front side of the LDPE-ITXSP film and then quartz plate B was placed onto the film to spread out the solution uniformly. Finally, this system was placed under the xenon lamp (filter was added with band pass of 380-700 nm,

irradiation intensity was 3 mW cm^{-2} at 420 nm) for 30 min at room temperature. After the grafting reaction, the the Control A film was washed with Tris-HCl buffer five times to remove the unimmobilized enzymes.

To prepare the Control B film, Solution A was cast onto the trypsin loaded film and then the system was sandwiched between two quartz plates. This system was placed under the same xenon lamp for 30 min at room temperature. After the grafting reaction, the Control B film was washed with the same buffer for five times to remove the unimmobilized enzymes.

2.3. Synthesis of target cytarabine

Firstly, the prodrug (1 mg) and the peptide with a sequence LTVSPWYGTGTQGTGRGTGDDR (4 mg) was dissolved into Tris-HCl buffer (4 mL, 0.2 M, pH=7.0) and then pre-warmed for 10 minutes. Secondly, a 4 cm $\times$ 4 cm DEL film was added into this solution. The catalytic reaction was performed in water bath with shaking at 35 °C. After 10 hours of incubation, the DEL film was taken out of the solution. Finally, a cellulose ester dialysis tube (nominal molecular weight cutoff = 500 Da~1000 Da) was used to remove the small molecule impurities in the solution, and then the solvent was removed by freeze drying. The crude product with a yield of 38 % was obtained and detected by MALDI-TOF/MS to verify the specific functions of the dual-enzymes.

3. Results and Discussion

As shown in Scheme 1, dual enzymes separately loaded film with two sides of cylindrical pattern could be fabricated by visible light induced graft polymerization. The heights of each PEG network pattern were characterized by the AFM measurement. Fig. 1(a) and 1(b) clearly showed that both patterns exhibited cylinder profile and the diameters were determined to be about 50 μm , in good accordance

with that of photomask. The height of trypsin entrapped pattern (upside) was $1.1\ \mu\text{m}$ and the height of TGase embedded cylinder (downside) was $1.0\ \mu\text{m}$, indicating the surface initiated graft polymerization of PEGDA in both side of LDPE-ITXSP proceeded in similar rate. And the results also confirmed that the two independent PEG patterns can be separately grafted onto both side of LDPE film simultaneously by visible light induced graft polymerization.

Fig. 1. Typical AFM images of (a) upside (LDPE-g-P(PEGDA)/Trypsin) and (b) downside (LDPE-g-P(PEGDA)/TGase) patterns of DEL film (the structure of

DEL film can be seen in Scheme 1b). Trypsin (0.43 mg) was immobilized on one side of the film and TGase (0.15 mg) was immobilized on the other side of the film

To further verify the successful encapsulation of two enzymes, the chemical composition of the DEL film was characterized by XPS. In Fig. 2(a), the C 1s core-level spectrum of LDPE-g-P(PEGDA)/Trypsin could be divided into five peaks: C-H and C-C (284.6 eV), C-O (286.2 eV), C=O (288.7 eV), C-N (285.9 eV) and O=C-N (287.4 eV) [40]. Among them, C-N and O=C-N were associated with the amino and peptide bonds in trypsin. The same peaks were found in TGase embedded layer in Fig. 2(b). Additionally, N 1s signal (399.5 eV), which was associated with covalently bonded nitrogen, could be found in Fig. 2(c) and 2(d). On the contrary, no N 1s signal was detected on P(PEGDA) surface (Fig. S5(a)). The above results proved that trypsin and TGase had been successfully immobilized on both side of the LDPE film.

Fig. 2. XPS C 1s and N 1s core-level spectra of DEL film. (a, c) Upside of the film (LDPE-g-P(PEGDA)/Trypsin) and (b, d) downside of the film (LDPE-g-P(PEGDA)/TGase). Trypsin

(0.43 mg) was immobilized on one side of the film and TGase (0.15 mg) was immobilized on the other side of the film

One key factor need to be seriously considered in enzyme immobilization is to ascertain that no enzyme leaked out during practical use. Because the non-swelling and uniform small mesh of PEG network and the relative large size of TGase (76.6 kDa) and trypsin (24 kDa), most of the two enzymes could be able to firmly constrained into the network without leakage. To verify this inference, Fig. 3 shows the release behavior of immobilized TGase and trypsin. For TGase, the amount of unimmobilized enzyme, which was collected and detected after washing process, was about 5 %. When the TGase loaded film was incubated in Tris-HCl buffer (0.1 M, pH=7.0), a release occurred during the first 10 h which may be caused by some defects of the networks. Then no further release could be detected in the case of prolonging the incubate time to 72 h. And totally about 3 % of the encapsulated TGase diffused out from the PEG network. Similar results can be found for trypsin loaded film. At first, about 8 % of absorbed trypsin was rinsed off after the grafting polymerization. And finally, about 1 % of trypsin leaked out after the film being incubated in Tris-HCl buffer for 72 h. Overall, more than 97 % of the immobilized two enzymes can be completely preserved in the PEG network grafted on LDPE film.

Fig. 3. Release behavior of the immobilized trypsin and TGase in PEG networks grafted on LDPE film. Two LDPE films were loaded with 0.15 mg TGase and 0.43 mg Trypsin, respectively

The activities of the two immobilized enzymes were another important factor to evaluate the performance of this catalytic film. The activity of trypsin was tested according to the method of Schwert and Takenaka [41]. And the activity of TGase was tested according to the method of Folk [42]. As shown in Fig. 4, the relative activity in single-enzyme loaded film group was defined as 100% (Trypsin 7774 U/mg and TGase 1961 U/g). In order to reflect the advantages of our strategy, the activities of dual-enzymes mixedly immobilized film (Control A) were also investigated. The relative activities of TGase on DEL film and Control A were found to be 83% and 63%, respectively. And the relative activities of trypsin on DEL film and Control A were found to be 97% and 77%, respectively. The results demonstrated that the separately immobilizing strategy in this work can avoid the interference of the two enzymes, thus increase their activities in the practical use.

Fig. 4. The activities of trypsin and TGase immobilized by different methods. Trypsin Loaded film was loaded with 0.43 mg Trypsin on one side of the film. TGase Loaded film was loaded with 0.15 mg TGase on one side of the film. In DEL film group, 0.43 mg Trypsin was immobilized on one side of the film and 0.15 mg TGase was immobilized on the other side of the film. In control A group, 0.43 mg Trypsin and 0.15 mg TGase were mixed together and immobilized on both sides of the LDPE film. In control B group, 0.43 mg Trypsin was immobilized firstly and then 0.15 mg TGase was immobilized on the Trypsin loaded layer. The activity of trypsin was tested in the reaction mixture consisted of N^α-benzoyl-L-arginine ethyl ester (BAEE, 1 mM) and Tris-HCl buffer (0.1M, pH=8.0). And the activity of TGase was tested in the reaction mixture consisted of hydroxylamine hydrochloride (0.1 M), N α -CBZ-Gln-Gly (0.03M), reduced glutathione (0.01M), CaCl₂ (5 mM) and Tris-HCl buffer (0.1 M, pH=6.0)

To demonstrate the superiority of our strategy, a sequential immobilization film (Control B) was prepared for comparison. For the fabrication of Control B film, a trypsin immobilized layer was first prepared by visible light induced graft polymerization. Due to the living feature of this technique, there are still dormant groups on the surface of the first layer. Thus another graft polymerization could be carried out to immobilize TGase into the second layer. As shown in Fig. 4, the relative activities of trypsin and TGase in Control B group were 83% and 73%, respectively. While in DEL film group, the relative activities of trypsin and TGase were 97% and 83%, which were higher than that in Control B group. Two reasons can explain this difference: 1) the contact of the two mutual inhibition enzymes in the interface in Control B may affect their activities; 2) the diffusion of the substrate in the bottom layer may be restricted by the upper layers, leading to a greater decrease of activity of trypsin.

Fig. 5. Operational stability of (a) trypsin and (b) TGase in DEL film at 35 °C. The activity of trypsin was tested in the reaction mixture consisted of N^α-benzoyl-L-arginine ethyl ester (BAEE, 1 mM) and Tris-HCl buffer (0.1M, pH=8.0). And the activity of TGase was tested in the reaction mixture consisted of hydroxylamine hydrochloride (0.1 M), N α -CBZ-Gln-Gly (0.03M), reduced glutathione (0.01M), CaCl₂ (5 mM) and Tris-HCl buffer (0.1 M, pH=6.0)

The ultimate goal to immobilize enzymes is to make them reusable and save the cost. Therefore, the operational stability of DEL film was investigated. As shown in Fig. 5(a), the immobilized trypsin could retain 92% activity after 6 cycles of use, and there was still 59% of the initial activity remained after 10 batches. In Fig. 5(b), the activity of TGase was 1399 U/g (86% of the initial activity) after the first 4 cycles of utilization. Then its activity decreased to 426 U/g (26% of the initial activity) after 10 cycles. Both of the immobilized trypsin and TGase showed better operational stabilities than other papers reported [43-45]. Overall, this DEL film can be repeatedly used for four cycles without obvious activity decrease.

Fig. 6. (a) Schematic route of synthesizing the target cytarabine, (b) MALDI-TOF/MS of the target peptide, product catalyzed by trypsin loaded film (green line), and the target cytarabine catalyzed by DEL film (blue line)

To verify that the two enzymes in this DEL film can work well together, a two-step reaction to synthesize a novel target anti-tumor drug was designed which was shown in Fig. 6(a). A peptide with sequence of LTVSPWYGTGQGTGRGTGDDR was customized as precursors. In this peptide, LTVSPWY is the target region (it has been demonstrated that this peptide can specifically bind to cancer cells but not interact with normal cells) [46, 47], R is the trypsin cleavable site, Q is the site for

TGase connection, GTGs are spacers and DDR can improve the aqueous solubility of peptide. When prodrug and LTVSPWYGTGQGTGRGTGDDR peptide were mixed and the DEL film was added, TGase could catalyze the conjugation of amine (with six carbon chains) in prodrug with Q of the peptide, and at the same time the hydrophilic GTGDDR moiety was cleaved by trypsin. Consequently, the final product could be obtained.

MALDI-TOF/MS was used to trace this two-step catalyzed reaction (Fig. 6(b)). The pure target peptide was first subject to MALDI-TOF/MS, and the MALDI-TOF/MS of the target peptide showed a species at 2282.1 (red line), which was in agreement with the expected target peptide $C_{96}H_{148}N_{30}O_{35}$ (calculated, $M=2282.1$ Da). To further demonstrate the functional mechanism of each enzyme in this two-step reaction, trypsin loaded film without TGase was used to catalyze a mixture of the target peptide and prodrug, the MALDI-TOF/MS of the catalyzed product gave a species at 1680.7 for $[M+H]^+$ (green line) which met our expectation that the immobilized trypsin can only remove the hydrophilic residues of the target peptide (calcd for $C_{74}H_{113}N_{21}O_{24}$, $M = 1679.8$ Da). In another group, a DEL film was added into the solution which contained a mixture of the target peptide and prodrug. After the reaction, the final product was characterized by MALDI-TOF/MS, and the MALDI-TOF/MS of this product showed a species at 2019.6 for $[M+H]^+$ (blue line), which was in accordance with the expected target cytarabine $C_{89}H_{133}N_{24}O_{30}$ (calcd for $M = 2018.9$ Da). Compared with the MALDI-TOF/MS result of the trypsin catalyzed group, the MALDI-TOF/MS result of the DEL film group can clearly prove that TGase can connect the prodrug and the target peptide together. In conclusion, the results demonstrated that the dual-enzymes in DEL film can worked well together.

4. Conclusions

A new protocol was developed to separately encapsulate TGase and trypsin simultaneously in PEG networks grafted on two sides of LDPE film by visible light induced graft polymerization. The results proved that more than 90% of the two enzymes can be successfully encapsulated onto LDPE film, and this DEL film showed good operational stability at the first four batches. Compared with the mixing co-immobilization strategy or the sequential immobilization strategy, the DEL film showed higher activities on both enzymes. Finally, to demonstrate the feasibility of this strategy, the DEL film was used to catalyze the synthesis of the target cytarabine. MALDI-TOF/MS analyses supported the successful obtaining of target product and demonstrated that the two enzymes can work well in a two-step reaction. It is believed that this simple and effective dual-enzymes separate immobilization strategy can establish a powerful platform for potential biomedical and industrial applications.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data:

Supplementary data associated with this article can be found, in the online version.

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Supplementary Information

Simultaneously and Separately Immobilizing Incompatible Dual-Enzymes on Polymer Substrate *via* Visible Light Induced Graft Polymerization

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Materials and Methods

Low density polyethylene (LDPE) film with a thickness of 50 μm was purchased from Beijing Plastic Factory No.7. Polypropylene (PP) non-woven fabric was ordered from Langfang Huijing Paper Plastics Manufacture Inc. The fabric and film were washed with excess acetone and then dried in a vacuum oven at 30 °C. Isopropyl thioxanthone (ITX) was obtained from TH-UNIS Insight Co., Ltd. Cytarabine came from TCI (Tokyo, Japan). Lipase from *Thermomyces lanuginose* (TLL, EC 3.1.1.3), trypsin from *bovine pancreas* (EC 3.4.21.4), transglutaminase from *guinea pig liver* (TGase, EC 2.3.2.13), poly(ethylene glycol) diacrylate (PEGDA) with molecular weight of 575, Boc-6-aminohexanoic acid, N α -CBZ-Gln-Gly, reduced glutathione and N α -benzoyl-L-arginine ethyl ester (BAEE) were obtained from Sigma-Aldrich Chemical Co. Peptide with the sequence LTVSPWYGTGTQGTGRGTGDDR was ordered from chinapeptides Co., Ltd. Other chemicals were purchased from Alfa Aesar Chemical Co.

Trypsin Activity Assays: The activity of trypsin was tested according to the method of Schwert and Takenaka's [S1]. The reaction mixture consisted of N α -benzoyl-L-arginine ethyl ester (BAEE, 1 mM) and Tris-HCl buffer (0.1M, pH=8.0). The catalytic reaction was performed under at 35 °C, and the hydrolysis of BAEE was

determined spectrophotometrically at 253 nm. The enzymatic activity (U) was defined as the formation of 1 μmol of N ^{α} -benzoyl-L-arginine per minute under the above conditions.

TGase Activity Assays: The activity of TGase was tested according to the method of Folk [S2]. The reaction mixture consisted of hydroxylamine hydrochloride (0.1 M), N α -CBZ-Gln-Gly (0.03M), reduced glutathione (0.01M), CaCl₂ (5 mM) and Tris-HCl buffer (0.1 M, pH=6.0). The catalytic reaction was performed at 35 °C, and the formation of hydroxamate was determined spectrophotometrically at 525 nm. A unit of activity represented the formation of 1 μmol of hydroxamate per minute under the above conditions.

Ratio of Enzymes Immobilized on Films: The ratio of enzymes immobilized in PEG networks were detected according to Bradford's method [S3]. Full details about this procedure was described elsewhere. Single-enzyme loaded films were used to detect the immobilization ratio of each enzyme. All the enzymes unimmobilized after grafting reaction were thoroughly rinsed and collected to determine the unimmobilized amount. For testing the release behavior of enzymes, the single-enzyme loaded films were immersed into Tris-HCl in a shaking incubator. And the amount of enzymes (releasing amount) in buffer was analyzed after 72 h. The enzyme

concentrations after immersion were measured by monitoring its absorbance at 595 nm using a UV–vis Spectrophotometer. The concentrations of enzymes in the buffer solution were obtained from a calibration curve, and then the ratio of enzymes immobilized can be calculated.

Instruments and Characterization: Scanning electron microscopy (SEM, JSM-6701F from JEOL Japan Electronics Co., Ltd) and atomic force microscopy (AFM, CPII from VEECO Co.) was used to determine the morphology and the thickness of the film. The UV-vis spectra were characterized by an UV–vis spectrophotometer (U-3900H from HITACHI Co.). The chemical structures of the synthesis products were determined by ^{13}C NMR and ^1H NMR (Bruker AVANCE Digital 600 MHz nuclear magnetic resonance spectrometer, BRUKER Co.) at 150 and 600 MHz, respectively. Surface chemistry was probed by X-ray photoelectron spectra (XPS, ESCALAB 250 from Thermo Fisher Scientific Co.) with a monochromator. The FT-IR spectroscopy was recorded on Nicolet NEXUS 670 spectrometer (Thermo Nicolet Co., U.S.). Mass spectrometry (MS, UPLC/Premier from WATERS Co.) was used to detect the mass of the synthesis products. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS, autoflex II from BRUKER Co.) was used to determine the mass of the biomacromolecules.

Scheme S1 The chemical reaction of the visible light induced graft polymerization

Synthesis of the Prodrug

The process to synthesis of the prodrug (product **3**) was shown in Scheme S2. In the three-step reactions, lipase from *Thermomyces lanuginose* (TLL, EC 3.1.1.3) was used to catalyze the second step reaction to obtain product **2** by the regio-selective conjugation. By using the visible light-induced surface polymerization method, TLL was immobilized into PEG net-cloth on non-woven fabrics (Scheme S3), and then this catalytic fabric was used to conjugate cytarabine and product **1** by transesterification. This prodrug with a six carbon chains here had three advantages: 1) the six carbon chains amino was proved to be the most appropriate for the catalytic action of TGase [S4]; 2) the regio-selective acylation of cytarabine can increase its anti-tumor activity [S5]; and 3) the carbon chains can be used as spacer to separate the drug and peptide.

Scheme S2 Chemo-enzymatic synthesis route of the prodrug (product **3**)

Scheme S3 Schematic illustration of TLL immobilization process by using the visible light-induced surface polymerization method

Synthesis of Product 1: Boc-6-aminohexanoic acid (12 g), cupric acetate (0.01g) and mercury acetate (0.1 g) were added into vinyl acetate (100 mL), and then 200 μ L sulfuric acid was slowly added. The reaction mixture was stirred at 60 °C for 24 h and finally sodium acetate (1.5 g) was added to stop the reaction. The solvent was

removed under reduced pressure and the crude product was purified by silica gel chromatography with the mixture of ethyl acetate and petroleum ether (11/89, v/v) as an eluent, to obtain product **1**. ^1H NMR (400 MHz, CDCl_3 , δ): 7.29 (dd, 1H, =CH-), 4.87 (d, 1H; $\text{CH}_2=$), 4.60 (d, 1H; NH), 4.56 (d, 1H; $\text{CH}_2=$), 3.11 (d, 2H; $-\text{CH}_2-$), 2.39 (t, 2H; $-\text{CH}_2-$), 1.70 (dt, 2H; $-\text{CH}_2-$), 1.49 (m, 2H; $-\text{CH}_2-$), 1.46 (s, 9H; $-\text{C}(\text{CH}_3)_3$), 1.34 (m, 2H; $-\text{CH}_2-$); FTIR (KBr): 3370 (NH), 1725, 1714 ($\text{C}=\text{O}$), 1649 ($\text{C}=\text{C}$).

Fig. S1. ^1H NMR spectrum of **1** in CDCl_3

Synthesis of Product 2: Product **2** was synthesized by transesterification of cytarabine (0.0972 g, 0.4 mmol) with product **1** (500 μL) in the solvent (12 mL, pyridine : *n*-hexane = 3 : 1 v/v). After mixing all the reactants tighter, a 4 cm $\times$ 4 cm TLL loaded fabrics was added. The esterification was performed with shaking at 48 $^\circ\text{C}$ for 72 hours. And then TLL loaded fabrics was taken out to stop the reaction. The solvent

was removed under reduced pressure and then the crude product was purified by silica gel chromatography with the mixture of methanol and ethyl acetate (9/91, v/v) as an eluent. The product was obtained as a white powder. ^1H NMR (400 MHz, DMSO-d_6 , δ): 7.48 (d, 1H; H-6), 7.07 (d, 2H; NH_2), 6.75 (s, 1H; -NH-), 6.09 (d, 1H; H-1'), 5.67 (d, 1H; H-5), 5.62 – 5.48 (m, 2H; 2'-OH, 3'-OH), 4.24 (ddd, 2H; H-5'), 4.03 – 3.77 (m, 3H; H-2', H-3', H-4'), 2.89 (dd, 2H; $-\text{CH}_2-$), 2.33 (t, 2H; $-\text{CH}_2-$), 1.65 – 1.40 (m, 4H; $-\text{CH}_2-$), 1.38 (s, 9H; $-\text{C}(\text{CH}_3)_3$), 1.25 (m, 2H; $-\text{CH}_2-$); FTIR (KBr): 3200-3600 (NH, NH_2 , OH), 1728, 1690 ($\text{C}=\text{O}$).

Fig. S2. Representative MS spectra of the product **2**

Fig. S3. ^1H NMR spectrum of **2** in DMSO-d_6

As shown in Fig. S2, the molecular weight detected were 457.2 for $[\text{M}+\text{H}]^+$ and 479.1 for $[\text{M}+\text{Na}]^+$, which met the molecular weight of product **2** (calcd for $\text{C}_{20}\text{H}_{32}\text{N}_4\text{O}_8$, $M = 456.2$). The acylation site of cytarabine was also determined on the basis of its ^{13}C NMR spectrum. As shown in Table S1, the ^{13}C NMR spectrum of the product showed a downfield shift of 2.56 ppm on C5' compared with the same carbon atom in cytarabine, and the directly neighboring carbon atom (C4') gave an upfield shift of about 3.09 ppm due to the acylation of the hydroxyl group of C5'. In addition, peaks of $-\text{C}(\text{CH}_3)_3$, $-\text{CH}_2$ and $\text{C}=\text{O}$ appeared with the determinate chemical shifts. According to Yoshimoto et al [S6]., the acylation of a hydroxyl group of cytarabine results in a downfield shift of the peak corresponding to the O-acylated carbon atom, on the contrary, an upfield shift of the peak corresponding to the neighboring carbon atom. The result indicated that the acylation site of cytarabine was on C5'.

Table S1 ^{13}C NMR spectral data for cytarabine and product **2** (δ , ppm)^a

Carbon numbers	Cytarabine	Product 2
Base moiety		
2	155.16	155.55
4	165.53	165.56
5	92.32	92.51
6	142.83	142.80
Sugar moiety		
1'	85.82	86.13
2'	74.77	74.28
3'	76.37	76.74
4'	84.82	81.73
5'	61.16	63.72
Acyl moiety		
-CH ₂		24.11~33.35
C=O		172.75
-C(CH ₃) ₃		77.30
-C(CH ₃) ₃		28.24

^a All samples measured in DMSO-*d*₆.

Synthesis of Product 3: The *t*-butoxycarbonyl (Boc) group in product **2** was de-protected with HCl/EA. The de-protection was performed at 30 °C for 24 hours. After that, a centrifuge (8800 rpm.) was used to get precipitate from the solutions. The precipitate was washed with EA for three times and then the crude product was dried in a vacuum oven at 30 °C to obtain product **3**, with 83% yield. MS (ES+) *m/z*: [M+H]⁺ calcd for C₁₄H₂₃N₅O₆, 357.2; found, 357.3; [M+Na]⁺ calcd for C₁₄H₂₃N₅O₆, 379.2; found, 379.3.

Fig. S4. ^1H NMR spectrum of the crude product **3** in $\text{DMSO-}d_6$

Preparation and Characterization of TLL Loaded Film

In Situ Immobilization of TLL on Non-woven Fabric: TLL (300 μL) was mixed with PEGDA (1200 μL) firstly and then the solution was cast onto a PP-ITXSP fabric. The fabric was sandwiched between two quartz plates to spread out the PEGDA/TLL solutions. The time of the polymerization was 90 min under a xenon lamp (filter was added with band-pass of 380–700 nm, irradiation intensity was 3 mW cm^{-2} at 420 nm). This fabric was then washed with deionized water alternately three times to remove the unimmobilized TLL and PEGDA.

TLL Activity Assays: The enzymatic activity of immobilized TLL was detected by the method described previously [S7]. The hydrolytic activity of TLL was tested with olive oil emulsion containing 3% (w/v) PVA. A certain amount of immobilized TLL was added in 4 mL of the emulsion and 5 mL of the phosphate buffer saline (0.05 M, pH=7.4), and then the hydrolysis reaction was carried out at 40 °C for 15 min. The quantity of fatty acid produced was measured by titration with 0.05 M KOH solution. One unit of enzyme activity was defined as the amount of TLL loaded fabric that produced 1 μmol of fatty acids per minute under the above conditions. The activity of the TLL loaded fabric was found to be 880 U/g.

Fig. S5. SEM images of (a) PP non-woven fabrics and (b) TLL loaded fabrics

Fig. S5(a) and S5(b) show the SEM images of non-woven fabric, and TLL loaded fabric. The significant differences between Fig. S5(a) and 5(b) can clearly prove that the P(PEGDA)/TLL layer can be grafted onto non-woven fabric uniformly.

Fig. S6. XPS C 1s and N 1s core-level spectra of (a, c) Fabrics-g-P(PEGDA) and (b, d) TLL loaded fabrics

To further confirm the successful preparation of TLL loaded fabric, XPS was used to determine the chemical composition of the modified non-woven fabrics. Fig. S6 shows the C 1s and N 1s core-level spectra of fabric-g-P(PEGDA) (fabric grafted P(PEGDA) without TLL as control) and TLL loaded fabrics. In Fig. S6(a), the C 1s core-level spectrum of fabric-g-P(PEGDA) could be curved-fitted into three peak components with binding energies at 285.0 eV for the C–H and C–C species, 286.6 eV for the C–O species, and 288.8 eV for the O=C species, and the XPS analysis

showed that the oxygen-to-carbon signal ratio (O/C) was 0.46 which was equal to the theoretical ratio of 0.46 calculated from pure P(PEGDA). Thus, the results demonstrated that PEG networks had been successfully grafted onto non-woven fabrics. Beyond that, XPS also provided a feasible method to verify fabric-g-P(PEGDA) and TLL loaded fabrics. In Fig. S6(b), the C 1s core-level spectra of the TLL loaded fabrics surfaces possessed two new peaks at about 285.9 and 287.4 eV, attributing to the C–N and O=C–N species which were associated with the amino and peptide bonds in TLL. Additionally, a N 1s signal at binding energy of about 400.5 eV, characteristic of covalently bonded nitrogen, could be found in Fig. S6(d). Whereas, no N 1s signal was detected in fabric-g-P(PEGDA) surfaces (Fig. S6(c)). All the above results indicated that TLL had been successfully immobilized in PEG networks on PP non-woven fabrics.

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