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Layer-by-layer self-assembly of Cibacron Blue F3GA and lipase on ultra-fine cellulose fibrous membrane

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ABSTRACT

Cibacron Blue F3GA (CB) dye and lipase from *Candida rugosa* were assembled into multiple alternating bilayers on ultra-fine cellulose (Cell) fiber surfaces via electrostatic layer-by-layer (LBL) deposition. The presence and even coverage of CB and lipase molecules on the fibers were clearly evident by FTIR spectra and microprobe element mapping. The average thickness of each CB/lipase bilayer was ~11 nm and the total LBL thickness increased with increasing numbers of bilayers up to 5 bilayers. The CB and lipase loadings on the Cell fibrous membrane also increased with the increasing bilayers. While CB was loaded at ~8.1 mg/g Cell from layers 2–4, lipase loading was ~3.3 mg/g Cell for the first 2 then became more varied, i.e., between 1.5 and 6.5 mg/g Cell, from layers 3–5. The maximum catalytic activities of lipase bound in the CB/lipase bilayers were 97.0, 90.4, 85.2, 76.1, and 55.6 U/mg of lipase with 1–5 bilayers, respectively. The catalytic activity of lipase bound in all 5-bilayer LBL was equivalent to 45% of that of free lipase. The successful assembling of alternating layers of CB and lipase as shown by incrementally increasing thickness and loading demonstrate that LBL is a promising approach to immobilize enzymes as nano-films on 3D fibrous templates.

1. Introduction

Layer-by-layer (LBL) adsorption of oppositely charged species from aqueous media onto solid surfaces is a versatile approach to assemble nanometer-scale multi-layers on solid surfaces [1]. The simple charge requirement is applicable to a diverse range of species, enabling the incorporation of a wealth of different materials [2] while maintaining the inherent properties and structure of the solid supports [3]. Furthermore, the use of water is highly attractive from the environmental perspective as well as technical capability of incorporating biomolecules, such as proteins and DNAs. For instance, adsorption of charged proteins onto oppositely charged surfaces has been well known for constructing monomolecular layers [4], oriented super-molecular architecture for biological sensors [5] and polyelectrolyte multi-layer biosensors [6] as well as for multi-step chemical catalysis [7]. Moreover, polyelectrolytes with multi-functionality offer additional options in building the layered structure via hydrogen-bonding, step-by-step reaction, molecular recognition, bio-recognition, and charge-transfer interaction [8].

The LBL electrostatic approach has been demonstrated to bind enzyme proteins to fiber surfaces [5,8,9]. The LBL approach has many advantages over the common enzyme immobilization methods of physical adsorption, entrapment, chemical covalent attachment or crosslinking. Most significantly, the enzyme immobilization via LBL can be on any support materials as long as the surfaces can be charged and involve aqueous buffer as the media at moderate temperatures, highly conducive to nearly all enzymes. For instance, the negatively charged laccase enzyme (at pH 4.5) could be alternately assembled with positively charged poly(dimethyldiallyl ammonium chloride) (PDDA) into three 5–7 nm PDDA/laccase bilayers on bleached softwood cellulose fibers (20 μm diameter) [9]. Cationic dye 5,10,15,20-tetrakis(4-N-methylpyridyl)porphine tetra(p-toluenesulfonate) (TMPyP) and sodium salt of DNA from salmon spermary were alternatively deposited onto quartz substrates coated with positively charge poly(ethylenimine) (PEI) [5]. Even the less ionic anionic alizarin violet could be assembled with polycations by first anchoring the dye molecules to poly(allylamine hydrochloride) (PAH) via a physisorption process [8]. The added anchoring step was shown to eliminate dye loss during subsequent polycation depositions. Therefore, anchoring low charge density or small molecules such as dyes and enzymes to polyions may facilitate effective deposition of homogeneous LBL self-assembled films.

We have previously covalently bonded a reactive dye ligand, Cibacron Blue F3GA (CB), to electrospun cellulose (Cell) fibers as affinity membranes for immobilizing lipase enzymes [10]. The bound lipase could be optimized by ligand concentration and ionic strength and exhibited 86% activity of the free

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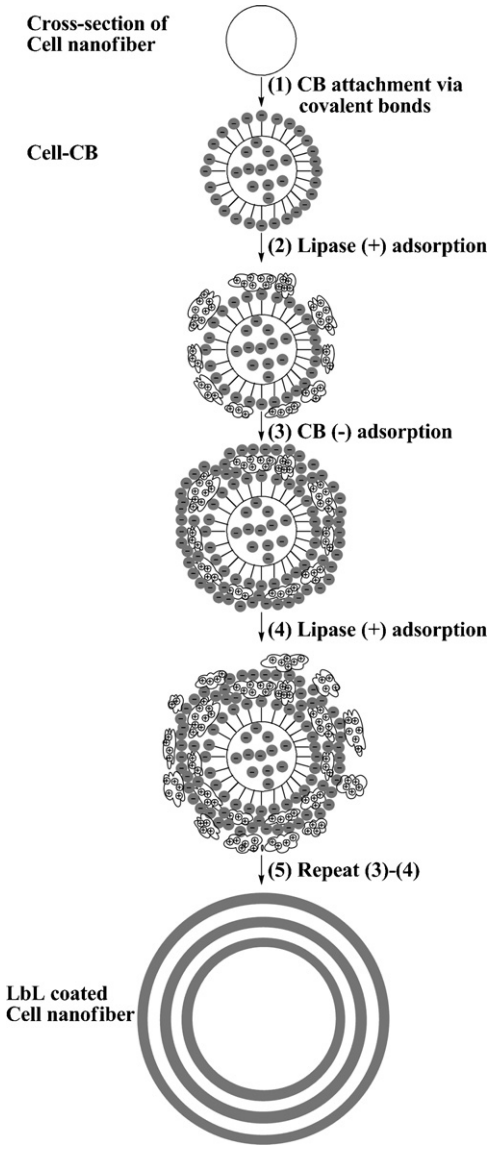

Fig. 1. Schematic diagram illustrating the LBL coating of the Cell fibers.

lipase. Various interactions including charge–charge interaction, hydrogen-bonding, hydrophobic effect were thought to be involved between the CB and lipase molecules. It is likely that these interactions may be sufficient for the LBL deposition of lipase onto the CB bound Cell fibers to improve enzyme loading and catalytic activity.

This study explores the LBL self-assembled approach to build up dye–enzyme multi-layers on the surface of ultra-fine Cell fibers. The high specific surface of the Cell template fibers and the nanometer-scale dye–enzyme multi-layers are expected to significantly enhance LBL loading of both molecules. The Cell nanofibrous membranes from electrospinning were chosen as the template for the LBL deposition of the anionic Cibacron Blue F3GA (CB) and cationic lipase from *Candida rugosa* as depicted in Fig. 1. The first layer of CB dyes was bound to Cell fibers *via* strong covalent bonds, involving nucleophilic substitution between the chloride of the triazine ring of the CB dye and the cellulose hydroxyl group under mild alkaline conditions [11]. With the three highly ionizable sulfonic groups, the CB dye molecules could be readily deprotonated to be negatively charged under acidic conditions. The lipase protein molecules were kept positively charged in a pH 4 buffer, i.e., below its p*I*, to be easily adsorbed to the negatively charged CB dye molecules on the surface of Cell fibers to complete the first bilayer. The first true LBL bilayer began with the next dipping in CB where the fiber surfaces were reversed back to be negatively charged, allowing the second layer of lipase molecules to be deposited to complete the second bilayer. This latter alternating CB and lipase LBL deposition process was repeated to achieve the designated numbers of bilayers up to a total of 5.

2. Experimental

2.1. Materials

Cellulose acetate (CA) (39.8 wt% acetyl content, average M_n *ca.* 30,000 Da, Aldrich), acetone (ACS grade, EMD Chemicals Inc.), N,N-dimethylacetamide (DMAc) (EMD Chemicals Inc.), sodium hydroxide (NaOH) (ACS grade, EMD Chemicals Inc.), Cibacron Blue F3GA (CB) (Polyscience, Warrington, FL, USA), sodium chloride (NaCl) (Certified ACS, Fisher Scientific), sodium carbonate anhydrous (Na_2CO_3) (ACS grade, EMD Chemicals Inc.), acetate buffer (pH 4, VWR), lipase from *C. rugosa* (1104 units/mg solid, type VII, Sigma), acetic acid (glacial, EM Science). Quick Start Bradford Protein Assay Kit 1 was purchased from BioRad (Richmond, CA, USA) for protein analysis. Gum arabic powder, sodium deoxycholate (99%), copper (II) nitrate trihydrate, triethanolamine hydrochloride, and sodium diethyldithiocarbamate, all from Acros, and olive oil (MP Biomedicals, Solon, OH), stearic acid (99%) (Alfa Aesar, Heysham, Lancs, UK) were used for lipase activity assay. All water used in the experiments was purified by Millipore Milli-Q plus water purification system.

2.2. Fabrication of Cell nanofibrous membranes

The Cell nanofibrous membrane was fabricated by a previously reported method used in our lab [12]. CA solution (15 wt% in 2:1 (w:w) acetone/DMAc) was loaded into two 20 mL all-plastic disposable syringes (National Scientific) and fed by a syringe pump (KDS 200, KD Scientific, USA) at 1 mL/h through a metal needle (23 gauge inner diameter, BD Medical, Franklin Lakes, NJ). A 14.25 kV voltage by a DC power supply (ES 30-0.1 P, Gamma High Voltage Research Inc., Ormond Beach, FL, USA) was applied to charge the solution which splayed into finer jets and collected onto a grounded aluminum plate (30 cm × 30 cm) at a distance of 15 cm at 27 °C and 48% humidity monitored by a digital thermohygrometer (Fisher Scientific, Pittsburgh, PA, USA). The nanofibrous CA membrane was deacetylated in 0.05 M aqueous NaOH solution at ambient temperature for 7 days to regenerate into cellulose (Cell). The nanofibrous Cell membrane was then rinsed with water and dried under vacuum at ambient temperature for 48 h.

2.3. Cibacron Blue F3GA bonded cell membrane

The first layer of Cibacron Blue F3GA (CB) was covalently bonded to the Cell nanofibrous membranes via the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl groups of the Cell under alkaline conditions [11]. About 1 g dried Cell nanofibrous membrane was added into 100 mL 1 mg/mL dye solution. After the wetting of Cell nanofibrous membrane (30 min), the solution was heated to 60 °C by water bath. Then 12 g NaCl was added into the reaction mixture and temperature was maintained at 60 °C for 1 h. The temperature of the reaction mixture was then increased to 80 °C and 1.2 g Na_2CO_3 was added. The reaction was continued for 2 h at 80 °C. The nanofibrous membrane was extensively washed with warm distilled water until the washings became clear to yield Cell with covalently bonded CB (Cell–CB). The remaining CB solution and all the washing solutions were combined, diluted to 1000 mL and measured by a UV–vis spectrophotometer (Evolution

600, Thermo Fisher Scientific) at 610 nm using an established calibration. In each CB immersion, the quantity deposited was derived from the difference between the initial and final CB in the solutions and normalized by the mass of Cell template. All the measurements were triplicated.

2.4. Layer-by-layer (LBL) assembly of lipase and CB

The negatively charged Cell–CB nanofibrous membranes were immersed in 0.2 mg/mL cationic lipase solution (pH 4) for 15 min, air dried for 1 min then rinsed in pH 4 acetate buffers for 1.5 min three times to construct the first bilayer. Each subsequent bilayer was constructed by repeating the alternating immersions in the anionic CB and cationic lipase solutions; each immersion was followed by three 1.5-min rinses in pH 4 acetate buffers to remove loosely adsorbed CB or lipase and 1 min air drying. The rinses after adsorption of each dye or enzyme layer minimize contamination of the next dipping solution by loosely bound electrolytes or any non-specifically adsorbed molecules [13]. This CB immersion-buffer rinsing-lipase immersion-buffer rinsing cycle was repeated to reach the desired bilayers of CB/lipase [14]. The LBL coated Cell nanofibrous membranes were rinsed by diluted acetic acid (pH 4) and water to remove the residue sodium acetate salt from the acetate buffer in the membranes and vacuum dried at ambient temperature for 24 h before further characterizations and enzyme assay.

The deposited lipase was determined by measuring the initial and final amount of lipase in the enzyme solution and the washing buffers (Bradford protein assay using bovine serum albumin as the standard) [15]. The adsorbed CB was also estimated by the spectrophotometric method described earlier, i.e., measuring the initial and residual CB in the solutions. The LBL CB and lipase coated films were referred as $(CB/lipase)_n$, where n is the number of bilayers.

2.5. Characterization and measurement

The morphologies of the uncoated and coated Cell nanofibrous membranes were examined using a scanning electron microscope (SEM) (XL 30-SFEG, FEI/Philips, USA) operated at a 5 kV accelerating voltage. All samples were coated with gold (2 min) using a Bio-Rad SEM coating system. The fiber diameters were measured by randomly selecting 100 individual fibers using an image analyzer (analySIS FIVE, Soft Imaging System). Average total LBL thickness after each LBL deposition was derived as follows:

$$\text{total LBL film thickness} = \frac{\text{fiber diameter after LBL} - \text{cell fiber diameter}}{2}$$

The average bilayer thickness was calculated by the total LBL film thickness divided the number of bilayers. Fourier transform infrared spectra (FTIR) of the samples prepared as KBr pellets were recorded under transmission mode by a Nicolet 6700 spectrometer (Thermo Fisher Scientific, USA) from 4000 to 400 cm^{-1} by accumulation of 64 scans and with a resolution of 4 cm^{-1} under ambient condition. Sulfur mapping of a 50 $\mu m \times 50\ \mu m$ scan area was performed under vacuum at a 7 kV accelerating voltage for 20 min using a Cameca SX-100 electron microprobe (AMETEK Inc., Gennevilliers, France). The sample was coated with carbon.

2.6. Activity assay of lipase in self-assembled LBL

The catalytic activity of the adsorbed lipase on Cell nanofibrous membrane was assayed by a standard photometric method [16]. A stabilized olive oil emulsion suspension was prepared by mixing 5 g olive oil, 5 g gum arabic and 95 mL 0.89% NaCl solution for 30 min. The incubation mixture was prepared by mixing olive oil suspension, 10 mM deoxycholate, and 1 M pH 8.5 triethanolamine buffer at a 50:5:45 volume ratio. The sample with bound lipase (~3 mg) was added to 1.0 mL incubation mixture, incubated in a 30 °C water bath for 10 min then denatured by heating at 100 °C for 5 min. Then 5 mL chloroform and 2.5 mL copper reagents were added and mixed in a shaker for 20 min to extract the fatty acids liberated from the hydrolysis of olive oil to the chloroform layer in the form of copper complex. The mixture was centrifuged for 5 min at 3000 rpm to separate the water and chloroform phases. 2 mL from the chloroform layer was carefully extracted and mixed with 0.25 mL 11 mM diethyldithiocarbamate color indicator. The UV–vis absorbance of the solution was measured at 436 nm against the corresponding blank sample after 20 min incubation at ambient temperature. Lipase activity was expressed as unit (U) per milligram lipase (U/mg lipase), where 1 unit is defined as the equivalent to hydrolyze 1.0 μequiv. of fatty acid from a triglyceride at pH 7.7 and 30 °C in 1 h. The activity of the free lipase was also measured by the same procedure for the comparison. Three measurements were performed for each sample. The relative activities of bound lipase were derived by comparing to the free lipase with normalized activity of 1.

3. Results and discussion

3.1. Formation of CB and lipase LBL bilayers

In the LBL deposition process (Fig. 1), the first bilayer consisted of covalently bonded CB on the Cell fibrous membranes, then an adsorbed lipase layer on top. The total CB on the Cell nanofibrous membrane amounted to 54.5 mg CB per g of Cell. The substantial CB loading reflected the fact that dye molecules not only resided on the fiber surfaces but also diffused to the inside of the Cell fibers. While CB triazinyl chloride reacts nucleophilically with the cellulose hydroxyl groups, only the dye molecules on the fiber surfaces are responsible for the negative charges that attract the positively charged lipase molecules to the fibers. After the first layer of the covalently attached dyes and electrostatically adsorbed enzymes, the subsequent deposition of each layer was driven by electrostatic interactions.

The pHs of the dye and enzyme solutions as well as all the buffer rinses were kept at 4 for several reasons. First of all, lipase enzyme and CB must be appropriately charged to enable the electrostatic attraction between the alternating layers. The CB dye molecules, with a pK_a value of 0.8 [18], remain negatively charged from pH 1 to 12, thus are essentially negatively charged. At pH 4, below the isoelectric point (pI, pH 4.8) of lipase enzyme [17], lipase proteins are positively charged. In fact, the pH cannot be above the pI of the enzyme because deprotonation of the amino acid side group would introduce negative charges, leading to repulsion of the negatively charged CB molecules. The enzyme activity is also maintained at pH 4.

The adsorption of CB and lipase increased generally with increasing numbers of bilayers, indicating progressive building of the dye and enzyme layers (Fig. 2). This gives the first evidence that the LBL process is effective in alternating adsorption of the CB dye ligand and lipase enzyme and the dipping and rinsing conditions are appropriate for such electrostatic deposition. Closer examination of the adsorption data showed some variations among the layers. In addition to the significantly higher covalently bonded CB in the first step, the quantity in fifth bilayer was much lower than most of the previous ones. The CB adsorbed in the second to the fourth bilayers ranged from 7.5 to 8.7 mg/g of the Cell template. The averaged 8.1 mg/g showed relatively similar dye deposition from each previous enzyme adsorbed surfaces. The quantities of lipase deposited were also similar in the first 2 bilayers, i.e., 3.1

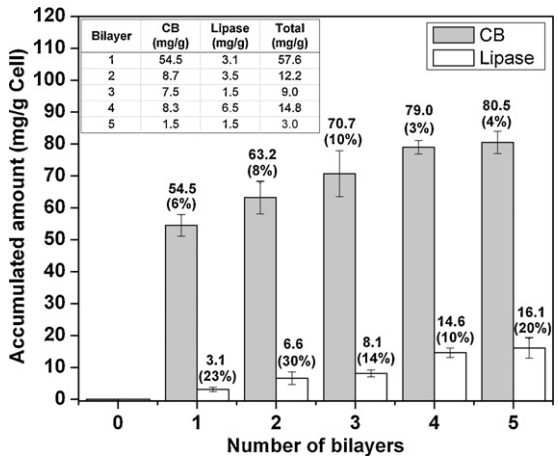

Fig. 2. Amount of LBL coated CB and lipase on the Cell nanofibrous membranes as a function of the number of bilayers.

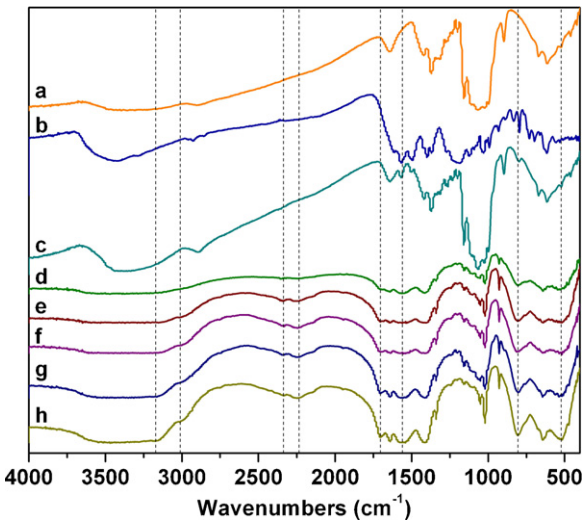

Fig. 3. FTIR spectra of (a) Cell, (b) CB, (c) Cell–CB, (d) (CB/lipase)1, (e) (CB/lipase)2, (f) (CB/lipase)3, (g) (CB/lipase)4, and (h) (CB/lipase)5.

and 3.5 mg/g, respectively, and then varied more significantly in third (1.5 mg/g) and fourth (6.5 mg/g) bilayers. The significantly lower quantities of both CB and lipase in the fifth bilayer suggest reduced effectiveness of the electrostatic interaction beyond the fourth bilayer.

The irregular loading of dyes and enzymes with the LBL coating process is not entirely unexpected due to the cycled dipping and washing process. The lipase loading from the second to the third bilayer was only 1.5 mg/g Cell, while the next CB loading of 8.3 mg/g Cell remained similar to the previous 2 CB layers. In fact the next layer of lipase, the fourth, was twice as high as the first 2. The mass loading data showed that the depositions of both dye and enzyme became irregular as LBL bilayer exceeds some threshold value. The constant increasing trend of enzyme loading, however, confirmed that LBL electrostatic attraction could be effective in improving enzyme adsorption to support matrix and small organic molecules such as dyes and biological macromolecules. Increasing the bilayer numbers might not improve the protein loading beyond a certain level. The irregular and non-uniform deposition resulted from the 3D membrane structure and deposition process was more evident in the third and fourth bilayers of deposited lipase. It should also be noted that other forces such as hydrophobic forces as well as affinity interactions between the dye ligand and enzyme protein may also be involved and possibly to greater extent. The synergic forces could play an important role in keeping a stable LBL supermolecular structure when the matrices became uncharged after drying.

3.2. Characterization of CB/lipase LBL on Cell membrane

The FTIR spectra of the Cell nanofibrous template exhibited peaks characteristic of cellulose, i.e., 3350 cm^{-1} of OH stretching, 2900 cm^{-1} of CH stretching, 1646 cm^{-1} of OH bending of adsorbed water, 1418 cm^{-1} of HCH and OCH in-plane bending, 1372 of CH deformation, and 896 cm^{-1} of COC, CCO, and CCH deformation and stretching (Fig. 3). The FTIR of CB showed protonated primary amine NH_3^+, S=O, and S–O at 2337, 1560, and 805 cm^{-1}, respectively. These CB characteristic peaks are consistent with reports by others [19,20]. The Cell with covalently bonded CB (Cell–CB) nanofibrous membrane showed both the characteristic peaks of Cell (3350, 1646, 1372, and 896 cm^{-1}) and CB (2337, 1560, and 805 cm^{-1}). The C–O–C bond formed from the nucleophilic reaction of the CB triazinyl chloride with the cellulose hydroxyl overlapped with C–O–C vibration at 1021 cm^{-1} and could not be clearly discerned. With increasing numbers of CB/lipase bilayers, all three CB characteristic peaks became stronger. New peaks at 3171, 3011, 2235, 1704, and 523 cm^{-1} from lipase also appeared, corresponding to the stretching vibrations of H-bonded N–H, COO–H of aspartate and glutamate residues, zwitterions of O–H, C=O of polypeptide, aspartate, and glutamate residues, $–CH_2–S–$ of cysteine and methionine, respectively [21]. Similar to CB, the lipase characteristic peaks also became increasingly stronger or broader with increasing numbers of bilayers.

The presence of CB and lipase in the LBL film coated fibrous membranes was also detected by elemental mapping of sulfur that is present in both CB dye and lipase enzyme (Fig. 4). The sulfur mapping spectra for the Cell fibrous template (Fig. 4a) appeared dark, consistent with the absence of sulfur, whereas CB–Cell with the covalently bonded CB showed evenly distributed sulfur across the membrane (Fig. 4b) as expected from the three sulfonic groups in each CB molecule. With the first layer of lipase, the sulfur signals further intensified (Fig. 4c), indicating the higher amount of sulfur from the cysteine and methionine in the adsorbed lipase. In addition, the appearance of blue signals in Fig. 4c could very possibly be due to the lower sulfur content in the enzyme than the dye. It appeared that the green signals representing the stronger sulfur density indicated the CB dye distribution whereas the blue signals associated with weak sulfur density from the lipase protein. Furthermore, the even distribution of blue and green signals in Fig. 4b and c suggests that both the dye and enzyme molecules are evenly distributed in the LBL coated films. The sulfur mapping together with the CB measurement, protein analysis, and FTIR spectra supports the successful deposition of CB and lipase on the Cell nanofibrous membranes.

3.3. Membrane and fiber morphology

The Cell fibrous template consisted of randomly oriented fibers with three-dimensional (3D) porous structures of micrometer size pores (Fig. 5a). The fiber and pore morphology remain largely unchanged with 1–3 LBL bilayers (Fig. 5b–d) while merging of some fibers became observable on membranes with 4 and 5 bilayers (Fig. 5e and f). Theoretically, the LBL coated films should be homogeneous monolayers because of repulsion of similarly charged molecules self-regulate the adsorption to a single layer. In practice, each additional deposition cycles may lead to surface flocculation and disturbance in layer uniformity [1]. Moreover, the competition of other forces, such as ionic and secondary interactions, during adsorption can cause additional variations in each of the layers which subsequently affect the next step in the process.

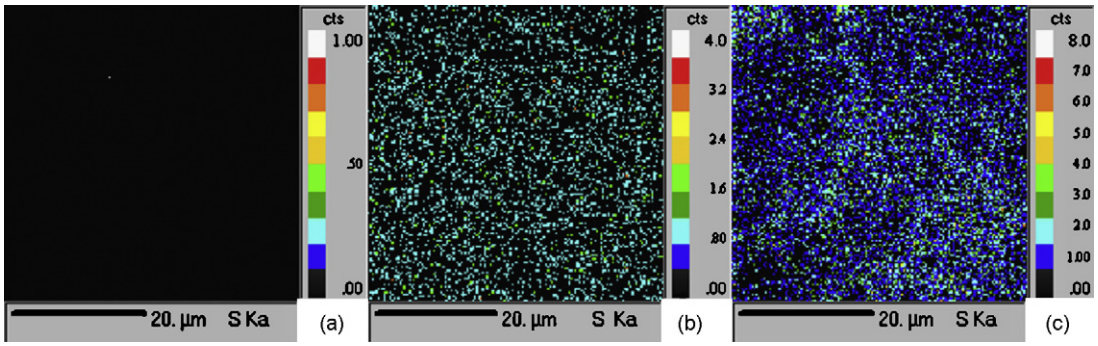

Fig. 4. Sulfur mapping of (a) pure Cell, (b) Cell–CB and (c) (CB/lipase)1 by electron microprobe.

The Cell fibers appeared cylindrical with an average diameter of 336 nm. After deposition of 1–5 CB/lipase bilayers, the fiber shape was retained, but the average fiber diameters increased progressively to 360, 381, 402, 420, and 437 nm, respectively (Table 1). The relatively large standard deviation of the original Cell fibers is common among electrospun fibers. The slight further increases in the standard deviations of the fibers with CB/lipase bilayers seem to reflect the irregularities in the bilayer which also increase with the numbers of bilayers except for the fifth bilayer. Nevertheless, the total average bilayer thicknesses increased consistently with the number of bilayers and were derived to be 12, 22, 33, 42, and 50 nm for the 1–5-bilayer coated Cell fibers, respectively. Furthermore,

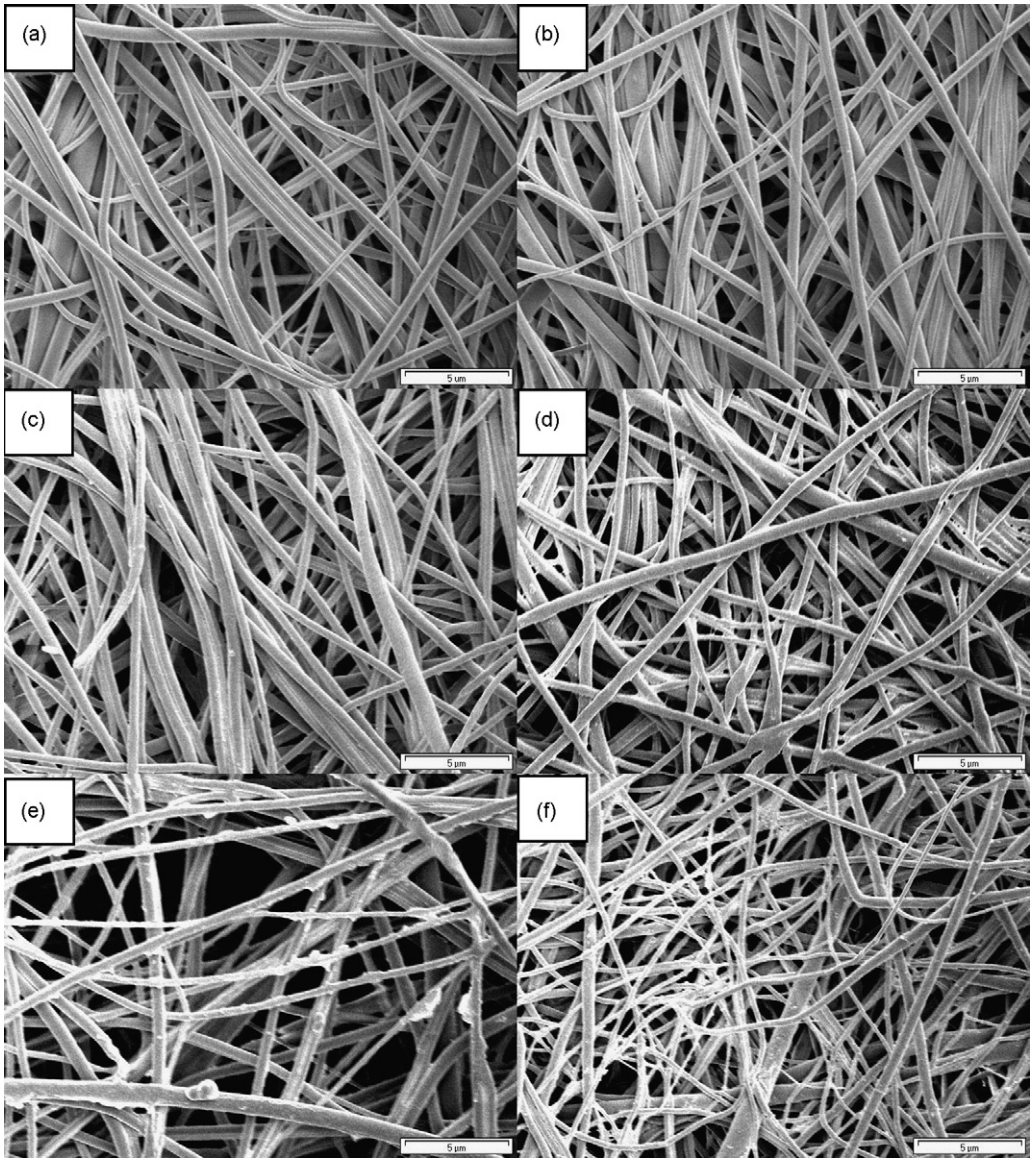

Fig. 5. SEM micrographs of Cell fibers coated with (a) 0, (b) 1, (c) 2, (d) 3, (e) 4, and (f) 5 bilayers of CB/lipase.

Table 1
Average diameters of pure and LBL coated electrospun Cell fibers.

Sample	Average fiber diameter (nm)	Standard deviation (nm)	Average total LBL thickness (nm)	Average bilayer thickness (nm)
Cell	336	99	0	0
(CB/lipase)1	360	116	12	12
(CB/lipase)2	381	113	22	11
(CB/lipase)3	402	124	33	11
(CB/lipase)4	420	144	42	10
(CB/lipase)5	437	128	50	10

the average thickness of each CB/lipase bilayer was estimated to be about 11 nm and this thickness was consistent for every additional bilayer.

3.4. Catalytic activity of LBL-bound lipase

The catalytic activity of LBL-Cell bound lipase, expressed as U per mass (g) of lipase, was monitored over a 4-h period. In this work, 1 U is defined as the ability to hydrolyze 1.0 μequiv. of fatty acid from a triglyceride at pH 7.7 and 37 °C in 1 h. All measured activities of the bound lipase at different times were extrapolated to 1 h for comparison. The lipase enzyme in all LBL deposited samples with 1–5 CB/lipase bilayers exhibited maximum activity within 10 min, then lowered with assay time (Fig. 6). This activity–time profile was identical to that of free, unbound lipase, indicating the surface nature of these bound lipase and supporting the advantage of the ultra-high specific fibrous template. The peak catalytic activity as well as the time profile of LBL-Cell bound lipase decreased with increasing numbers of LBL bilayers. The peak activities were 97.0 U/mg for lipase bound in the first layer and 90.4, 85.2, 76.1, and 55.6 U/mg for that bound in 2–5 bilayers, respectively. The catalytic activities of the lipase decrease by 42.7% with the increasing bilayer numbers from 1 to 5. Meanwhile, the released fatty acids increased greatly for the 1 bilayer sample in the 240 min assay process, indicating the continuous increase of the total activity of bound lipase. For membranes with 2–5 bilayers, the releasing of fatty acids slowed down and almost leveled after 60 min, possibly due to increasing difficulty in the diffusion of substrate and product from increased bilayer barriers. In comparison to free lipase, the relative activities of the LBL-bound lipase were 0.82, 0.74, 0.70, 0.63, and 0.45 for the cell membranes with 1, 2, 3, 4 and 5 bilayers, respectively (Fig. 6).

Several factors can contribute to the lowered catalytic activities of the LBL coated lipases. First, the small size of CB molecules may produce the more tightly packed CB layers which limit diffusion of both substrates and formed products. The products may have difficulties in diffusing from the compact CB/lipase layers to the reaction solution, inhibiting the formation of new products. This product inhibition effect might be the main reason for the dramatic decrease of catalytic activities of the inner LBL coated lipases. Secondly, the conformation of lipase proteins may be affected by surrounding CB molecules, becoming less favorable for catalysis. These conformational effects may include changes in surface properties, i.e., charge density and hydrophobicity. The catalytic similarities of the LBL coated lipases indicate that lipase enzymes on the outermost layer have higher accessibility by substrate than those reside in the inner layers. Therefore, majority of released fatty acid may be the catalyzed products of the enzymes on the outermost layer on the fibers. The specific activity of coated lipase decreased gradually with the increasing of bilayer numbers. However, the enzyme loadings and the total catalytic activities were improved with the increasing numbers of bilayers even though the enzyme catalytic activities of the inner layers were reduced accordingly.

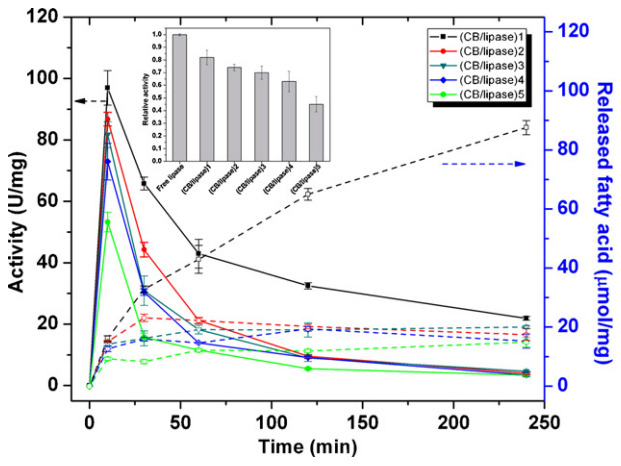

Fig. 6. Kinetics of catalytic hydrolysis (30 °C, pH 8.5) of olive oil by LBL coated lipase. The inset shows the relative catalytic activities of the free and LBL coated lipases (assay time: 10 min; pH 8.5; temperature: 30 °C).

4. Conclusions

An LBL self-assembly process was developed to assemble multiple and alternating layers of CB and lipase onto pure Cell fibrous membranes. Successive depositions of CB and lipase for up to 5 CB/lipase bilayers were demonstrated and confirmed by FTIR and sulfur mapping. Except the first layer of 54.5 mg/g Cell CB loading by covalent bonds, the subsequent 2–4 layer CB adsorptions averaged at a rate of 8.1 mg/g Cell and then dramatically decreased to 1.5 mg/g Cell in the 5th layer. Following a similar pattern as that of CB dye, the lipase adsorptions kept stable at a rate of 3.1 and 3.5 mg/g Cell for the first 2 layers but fluctuated at 1.5, 6.5, 1.5 mg/g Cell in third to fifth layers, respectively. Irrespectively of the order and number of bilayers nor loading, each bilayers had an average thickness of 11 nm. The free and bound lipase in the fibrous membranes with 1–5 CB/lipase bilayers reached the maximum activity within 10 min, indicating the surface nature of the bound enzyme and the advantages of the ultra-high surface template fibers. With the increasing bilayers, the maximum catalytic activities of LBL-bound lipases decreased from 97.0 to 55.6 U/mg, while the total catalytic activities as well as the lipase loadings increased. These results showed that LBL self-assembled dye–protein bilayers are versatile and effective approach for immobilizing enzyme molecules on fibrous templates. The structure and numbers of bilayers are critical to the catalytic efficiency of the bound enzymes.

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