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Ultra-high surface fibrous membranes from electrospinning of natural proteins: casein and lipase enzyme

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Natural proteins such as casein and enzymes cannot be processed into fibrous forms. With the addition of another polymer such as poly(ethylene oxide) (PEO) or poly(vinyl alcohol) (PVA), casein, a natural protein, was electrospun into ultra-thin fibrous membranes. Fibers in these membranes had diameters between 100 and 500 nm. These fibrous membranes were rendered insoluble by chemical crosslinking with 4,4′-methylenebis(phenyl diisocyanate) (MDI) in THF. The electrospinning method has also been utilized for enzyme immobilization on solid support. The lipase encapsulated in the ultra-fine fibrous membranes exhibits higher catalytic activity towards hydrolyzing olive oil than that in the cast films from the same solution.

1. Introduction

Electrospinning (ES) is a fiber-forming process by which either polymer solutions or melts are charged to high voltage to form fine jets. It was first reported in 1934 [1] and had received relatively little attention until the last decade. With the forthcoming of the "nano" technology era, electrospinning of more than 30 polymers, mainly synthetic ones, has been reported. Most of the studies have focused on the effects of electrospinning parameters on the morphology of the electrospun fibers [2–6]. For instance, the effects of polymer concentration [4, 5], solvent [6], salt [6], accelerating voltage [4], field strength [5], and deposition distance and time [5] on the structure and morphology of electrospun fibers have been studied using PEO, one of the most extensively studied polymers. The general conclusion is that fiber diameters may be reduced by either decreasing polymer concentration or increasing applied field strength. A few have studied the microstructure and properties of the electrospun PEO from solutions [4] and poly(ethylene terephthalate) (PET) and poly(ethylene naphthalate) (PEN) from melts [7].

What distinguishes electrospun fibers from naturally occurring and conventionally manufactured fibers are their much finer fiber sizes. The reported fiber sizes range from 40 to 2000 nm, but only a few can go down to tens of nanometers. The unique characteristics of electrospun fibers have led to the development of protective membranes [8], conductive fibers [9], porous fibers [10], reinforcing additives [11, 12] and template for fabricating nanotubes [13]. One of the advantages of electrospinning is its capability to process polymers that are not processable by conventional fiber spinning techniques. For intstance, DNA [14] and metal-organic polymer [15] fibers have been produced by electrospinning. Proteins are the major category of natural polymers that are difficult to be processed into fibrous forms. This is because of their complex macromolecular and three-dimensional structures as well as strong inter- and/or intra molecular forces. In recent years, advances in protein engineering have made available sequence-controlled protein polymers, which in term renew the interest in the conversion of these synthetic proteins into useful materials. Synthetic proteins [5, 16] produced by biotechnology have been processed into fibrous membranes by electrospinning. To our knowledge, electrospinning of natural proteins into fibrous materials has not been attempted.

This study investigates the feasibility of electrospinning in converting natural proteins into fibrous membranes. Electrospinning of casein, a milk protein, was first examined, then extended to include enzyme proteins. The goal was to explore if electrospinning of enzyme-containing solutions could physically immobilize enzyme molecules onto nanofiber membranes. It is thought that the large surface areas and fine porous structures of electrospun fibrous membranes should greatly increase the efficiency and the catalyzing ability of the immobilized enzymes.

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2. Experimental

2.1. Materials

Poly(ethylene oxide) (PEO) (Ave. M_v 600,000 dalton, Aldrich), poly(vinyl alcohol) (PVA) (Ave. M_w 124,000–186,000 dalton, Aldrich), casein (Acros), triethanolamine (98%, Aldrich) were used as received. The crosslinking agent 4,4′-methylenebis(phenyl isocyanate) (MDI) from Aldrich was distilled before using. Enzymes of lipase (type VII, from Candida rugosa) and α-Chymotrypsin (type II, from Bovine pancrease), both from Sigma, were used as received. The regents needed for lipase assay including olive oil (substrate), gum Arabic (emulsion regent), sodium deoxycholate (emulsion regent), triethanolamine hydrochloride (buffer), sodium diethyldithiocarbanate (color indicator) and stearic acid (standard), all from Acros, were used without further treatment.

2.2. Fibrous membrane formation

All polymers were dissolved in their respective solvents before mixing. Casein was dissolved in 5% (wt) aqueous triethanolamine at ambient temperature. PVA and PEO were dissolved in 50°C water with agitation overnight. The lipase was dissolved in 10 mM bis-tris propane buffer (pH = 7.8) at ambient temperature. The electrospinning solution was prepared by mixing individually prepared solutions at specific proportions to achieve desired compositions. For electrospinning, each solution was placed in a glass tube bearing a plastic pipette (Fisher, 0.5–10 μl). A stainless steel electrode was immersed in the solution and connected to a power supply (Gamma High Voltage Research Inc.). Grounded counter electrode was connected to the aluminum foil collector. Typically electrospinning was performed at 25 kV with a 30 cm distance between the tip of the pipette and the collector. The glass tube was tilted at a slight declining angle of 2–10° to form a bead of polymer solution at the pipette tip for initiating the jet. It usually took a few hours to obtain sufficiently thick and integrated membrane that can be detached from the aluminum foil collector. The membranes on aluminum foils were dried under vacuum at room temperature before they were detached. For comparison purpose, cast membranes from the same solutions as those used for electrospinning were prepared.

The ES membranes were crosslinked by immersing in 1% MDI in THF for 10 hrs, then washed consecutively in THF, acetone and water, and dried under vacuum at ambient temperature. To study the biodegradability and phase separation of the membranes, the crosslinked ES membrane was digested by α-chymotrypsin (25 mg/ml) in HEPES buffer (pH = 7.8) at 25°C for 4 days.

2.3. Enzyme activity

The catalytic activity of the lipase-containing ES membrane on hydrolyzing olive oil was assayed using a standard photometric method [17]. Briefly, a stabilized olive oil emulsion substrate and lipase immobilized membrane were added to a buffer (pH = 8.5) and incubated at 30°C under constant shaking (60 rpm). Following a designed assaying time (5 hrs for membrane-supported lipase; 10 min for free lipase), the incubation solution was heated to 80°C for 10 min to denature the lipase. Copper (II) sulfate aqueous solution and chloroform were added to extract the liberated fatty acids from hydrolysis of olive oil to chloroform layer in the form of the copper salts. The same procedure using denatured lipase was adopted to prepare a blank solution. The amount of copper(II) ion, or COO^-, was determined spectrophotometrically at 436 nm (HITACHI U-2000 Spectrophotometer) with sodium diethyldithiocarbamate as a color indicator. The absorbance was converted to concentration using a calibration from stearic acid standard solutions. The lipase activity was the amount of free fatty acid liberated per hour under assay condition. The enzyme-catalyzed hydrolysis over time was also evaluated. The activity of recycled membranes was also measured. The assayed membranes were thoroughly wahsed in water, dried in vacuum at ambient temperature, and stored at 4°C for 5 days before being assayed the second time.

2.4. Characterization

The thermal properties of the membranes were measured using a SHIMADZU DSC-50 differential scanning calorimetry (DSC) and a SHIMADZU TGA-50 thermogravimetric analysis (TGA) under N_2 at a 10°C/min heating rate. Fiber morphology and pore structure of the membranes were examined using an International Scientific Instrument model DS 130 scanning electronic microscope (SEM) at 10 kV. All samples were sputtered with gold.

3. Results and discussion

3.1. Casein ES membranes

In 5 wt% aqueous triethanolamine, casein could form viscous solutions at 10 to 30 wt% concentrations. However, none of these casein solutions could be eletrospun under all conditions allowed with the experimental setup. Significant die swelling, like the "Barus effect" in conventional fiber spinning, was observed at the tip of pipette, but could not sustain the formation of a stable liquid drop necessary to initiate jets.

Caseins are globular proteins whose molecular weights have been reported to be ranging from 19,007 to 25,230 daltons [18]. Over 55% of the amino acids in casein proteins contain polar side groups [19], i.e., 26%–COOH, 15%–NH_2 and 15%–OH. These polar groups enable the formation of intermolecular and intra-molecular hydrogen bonding among the casein protein molecules. The fact that casein solutions could not be electrospun is believed to be due to the high elasticity of the polymer, which is resulted from the globular structures and strong inter-molecular and intra-molecular forces of protein molecules. Therefore, if these inter-molecular and intra-molecular bonding could be reduced, the solutions should become electrospinable. Our approach to interrupt the 3-D structure and to dissociate the protein molecules was adding

TABLE I Electrospinning of PEO/casein and PVA/casein solutions

PEO:casein (w:w)	Spinning observation	Product
	(a) PEO/casein 5% total concentration	
0:100	Dripping	Liquid blot
5:95	Continuous	Non-fiber membrane, non-detachable
20:80	Continuous	Non-fiber membrane, detachable
40:60	Continuous	–
50:50	Continuous	Irregular fiber (w/ bead) membrane, detachable
60:40	Continuous	–
80:20	Continuous	Fine fiber membrane, detachable
100:0	Continuous	Fine fiber membrane, detachable
	(b) PEO/casein 10% total concentration	
0:100	Dripping	Liquid blot
20:80	Continuous	Fine fiber membrane, detachable
40:60	Heave jet, blocked	–
50:50	Jet radiation	–
80:20	Too viscous, no jet	–
	(c) PVA/casein 10% total concentration	
30:70	Dripping, blocked	Irregular fiber membrane, non-detachable
50:50	Dripping, partially blocked	Fine fiber membrane, detachable
70:30	Partially blocked	Fine fiber membrane, detachable
100:0	Partially blocked	Fine fiber membrane, detachable

[a] 5% PEO in water mixed with 5% casein in 5% aq. triethanolamine.
[b] 10% PEO in water mixed with 10% casein in 5% aq. triethanolamine.
[c] 10% PVA in water mixed with 10% casein in 5% aq. triethanolamine.

another polymer which has a dissimilar structure and capacity to form secondary bonding with proteins.

Aqueous solutions of either PEO or PVA were added to the casein solution at different mixing compositions to examine their abilities to dissociate the protein molecules. PEO, one of the most easily electrospun polymers, was added to casein from 5:95 to 80:20 PEO/casein ratios, first at a 5% total polymer concentration (Table Ia). The 5% pure PEO solution was electrospun to fine fibers with diameter generally below 500 nm (Fig. 1a). In replacing 20% PEO with casein, or at 4:1 PEO/casein, the mixture could be electrospun into fine fibrous forms (Fig. 1b). The diameters of fibers are between 500 nm and 1 μm. At a 1:1 PEO/casein mix, the electrospun fibers become irregular, reduce in sizes, and are interspersed with large beads (Fig. 1c). Increasing casein in the solution mixture to 80% resulted in mostly interconnected beaded structure with little fibrous structure (Fig. 1d). The PEO/casein soluton containing 95% casein generated essentially a non-fibrous membrane (Fig. 1e). The 5% PEO solution has a viscosity of 2791 centipoise (cP) which is much more viscous than the 5% casein solution (1.6 cP). As PEO is replaced by casein, the solution viscosities decreased and the morphologies of ES membranes changed from fibrous to beaded, then became completely non-fibrous. This is consistent with observations that electrospun membranes of PEO changed from filamentous membranes to beaded coating with filamentous/beaded intermediate stages when the solution viscosity was lowered by decreasing polymer concentrations or solvent viscosities [4, 6].

When the total polymer concentration was raised to 10% (Table Ib), the polymer solutions become more viscous, thus higher proportions of the less viscous casein solution can be incoporated while maintaining viscosities sufficiently high for electrospinning. The ES membrane from the 10% 20/80 PEO/casein solution shows a uniform and completely fibrous structure (Fig. 1f). This is in contrast to the interconnected beaded morphology generated from the 5% solution with the same PEO/casein compositions (Fig. 1d). In fact, if the PEO content exceeds 20% at 10% concentration, the viscosity of the PEO/casein solution increases dramatically and become difficult to process.

The 10% PVA aqueous solution, with a viscosity of 1239 cP, can be electrospun alone as well as when PVA is replaced with 30% to 70% casein (Table Ic). Fine fibrous membranes of uniformly sized fibers were generated from solutions containing up to 50% casein (Fig. 2). As casein reaches 70% and above, solution viscosity became too low and the fibers formed were irregularly shaped. The main distinction between PVA and PEO is that casein may be electrospun at varying contents of 30% to 70% in PVA. The maximum casein content in PVA which permits electrospinning and fiber formation is slightly lower than the 80% with PEO. Additionally, electrospinning of PVA/casein solutions is not as continuous as that of PEO/casein solutions. The pipette tip was blocked after 10 to 20 min and required frequent cleaning to resume the electrospinning process.

The as-spun fibrous membranes from PEO/casein and PVA/casein mixtures are instantaneously soluble in water. These membranes remain water soluble with further drying and storage. Since casein is insoluble in water, the instant water solubility of these casein-containing membranes suggests that casein molecules are thoroughly mixed and fully integrated with PEO and PVA. In order for these membranes to stay intact as solids in any aqueous environment, they have to be rendered insoluble in water first.

Diisocyanates are highly reactive toward both amines and alcohols and are capable of crosslinking by reacting

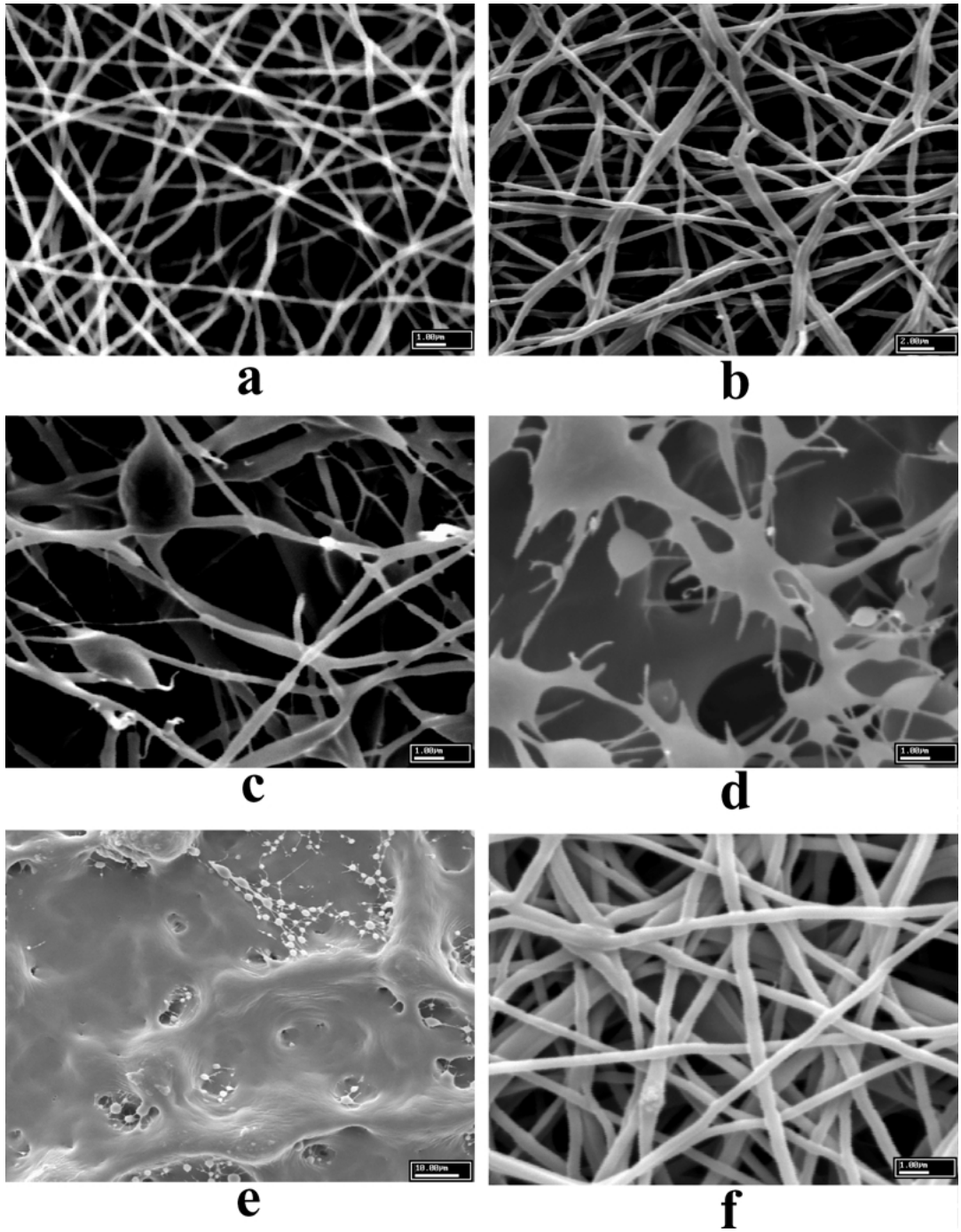

Figure 1 Electrospun membranes from PEO/casein solutions at concentration of 5% (Table Ia) for: (a) 100:0, (b) 80:20, (c) 50:50, (d) 20:80, (e) 5:95, and (f) 20:80 at 10% concentration (Table Ib).

with the amines in casein, the hydroxyls in PVA as well as the hydroxyl ends of PEO. Following crosslinking with the diisocyanate in THF, the porous characteristics of the PEO/casein (Fig. 3a, b) and PVA/casein (Fig. 3c) membranes have been retained. Crosslinking of the 20/80 PEO/casein (Fig. 3b) and 50/50 PVA/casein (Fig. 3c) membranes cause the fibers to become slightly relaxed and more densely packed in comparison to their uncrosslinked forms (Figs 1f and 2c, respectively). The effects may be cauesd by fiber swelling in the organic solvents and water used in crosslinking and rinsing, respectively. For the 50/50 PEO/casein membranes, on the other hand, crosslinking caused deformation (Fig. 3a) of the original fibrous and beaded forms of (Fig. 1c). Since each PEO molecule has only two –OH reactive groups at the chain ends, crosslinking among the casein molecules is expected to far exceed that among PEO in the 50/50 PEO/casein membrane. The deformation of the crosslined 50/50 PEO/casein membranes is likely a result of low crosslinking ability and higher content of PEO in this membrane. The retention of PEO in the 50/50 PEO/casein membrane is due to strong secondary forces between the PEO and casein rather than the crosslinking of PEO. In the crosslinked 20/80 PEO/casein membrane, the retention of fibrous form and mass after thorough wash in water indicates more highly crosslinked fibers and better retention of PEO. Additional evidence of PEO retention will be presented later in the FTIR and TGA analysis sections.

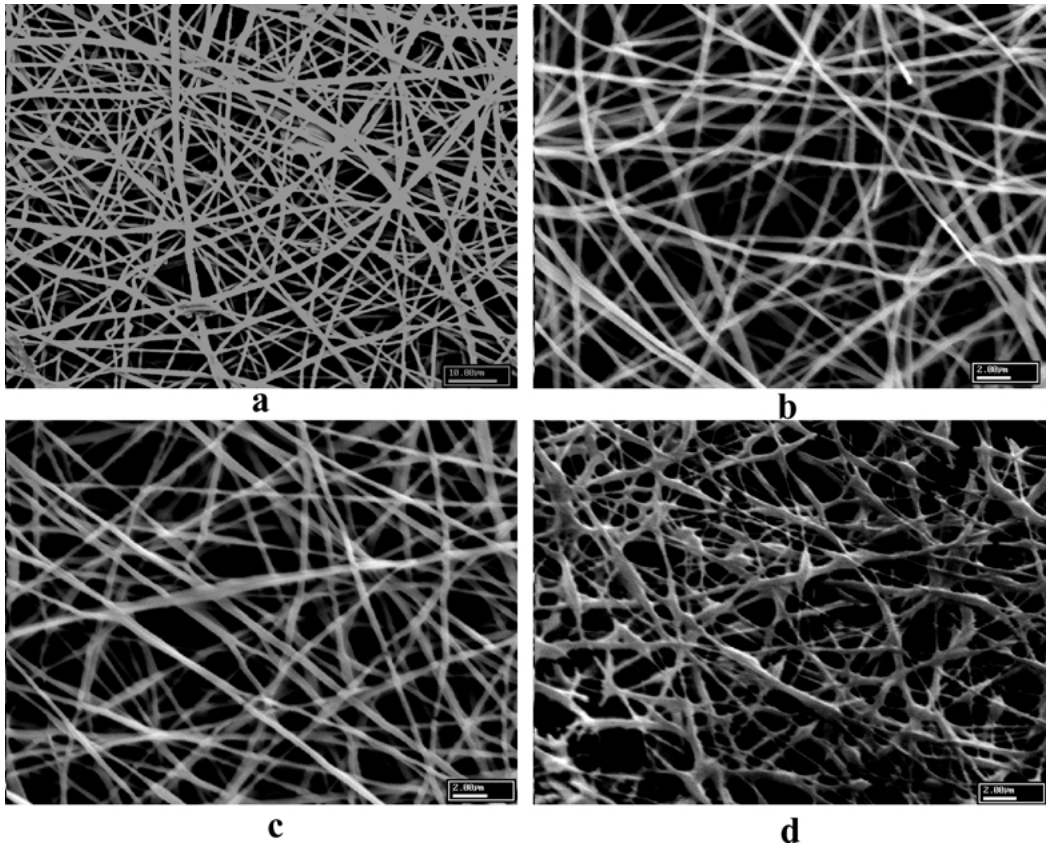

Figure 2 Electrospun membranes from 10% PVA/casein solutions (Table Ic): (a) 100:0, (b) 70:30, (c) 50:50, and (d) 30:70.

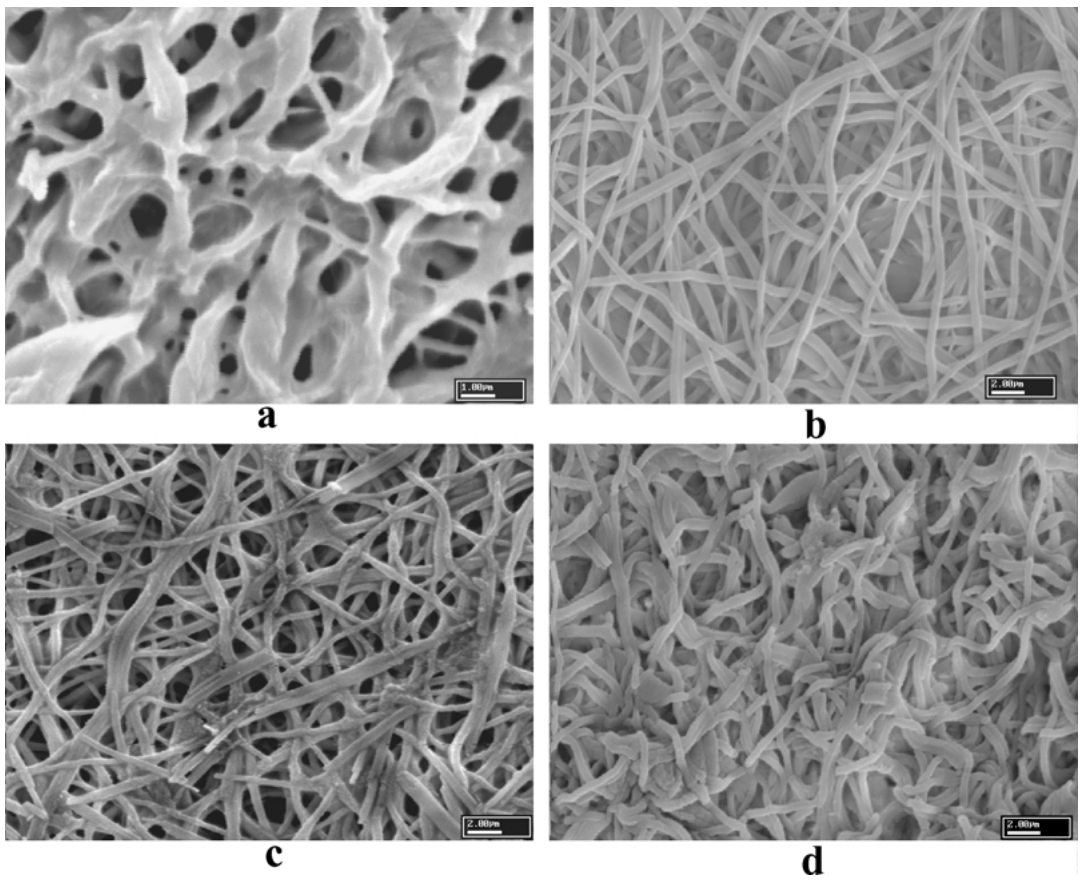

Figure 3 Crosslinked ES membranes of (a) 50:50 PEO/casein, 5%, (b) 20:80 PEO/casein, 10%, (c) 50:50 PVA/casein, 10%, and (d) a-chymotrypsin digested 3-b for 25 hr.

The insolubility in water and the retention of shape and mass of the membranes indicate the absence of macroscopic phase separation between casein and PEO or PVA. In another words, there are no large domains of PEO or PVA in these PEO/casein or PVA/casein fibers. Otherwise, the membrane shall fall apart in water after either PEO or PVA is dissolved. Furthermore, digestion of casein was attempted with the use of a protease, namely α-chymotrypsin. Following a 25-hr digestion, the fibers appear more relaxed, but the mass and the fibrous form remain (Fig. 3d). In fact, these membranes retain their forms even after 4 days of digestion. Therefore, retention of fibrous forms following dissolution of either components in these crosslinked casein membranes support the high compatibility between casein and PEO or PVA polymers in the electrospun fibers.

3.2. Structure analysis

The structural characteristics of the electrospun fibrous membranes from PEO/casein and PVA/casein mixtures can be further discerned by their thermal behavior. The solvent cast PEO exhibits a T_m at 70.7°C with a ΔH_f of 161.1 J/g (Fig. 4). The electrospun PEO membrane has a similar T_m, but a higher ΔH_f of 168.9 J/g, indicating higher structural order of crystallinity and/or orientation. The crude casein powder shows a moisture endothermic at 100°C and several overlapping decomposition peaks between 200°C and 350°C. With the addition of casein, both T_m and ΔH_f of the PEO portion in the electrospun PEO/casein membranes are lowered, suggesting reduced crystallinity and crystal sizes of the PEO domains in the mixed polymer membranes. At 20%, 50%, and 80% of casein, the T_m was reduced to 64.9°C, 62.6°C, and 57.0°C and the ΔH_f values were lowered to 128.1, 149.0, and 95.6 J per gram of PEO, respectively. An exothermic decomposition peak also appeared in presence of casein and increased in size with increasing amount of casein.

With 20% PEO, the ES membranes from 5% and 10% PEO/casein solutions exhibit similar thermal behavior, suggesting the same phase structure. Although these two membranes have completely different porous structures, i.e., beaded from the 5% (Fig. 1d) and fibrous from the 10% (Fig. 2b), phase structure in these electrospun PEO/casein membranes appears to be associated with the polymer compositions, but not their solution properties nor electrospun structures.

The solvent cast PVA has a T_m of 186.8°C, ΔH_f of 37.5 J/g and decomposes between 250°C and 350°C (Fig. 5). The electrospun PVA has a slightly higher T_m (188.7°C) and much increased ΔH_f (44.6 J/g). With the addition of casein, both the T_m and ΔH_f of PVA lowered, similar to the observations with PEO. At 30%, 50%, and 70% casein, the T_m of PVA in the membranes lower to 185.8°C, 180.6°C, 178.5°C, respectively, and the ΔH_f decrease to 24.3, 22.6 and 15.0 J per gram of PVA, respectively.

Comparisons between the cast films and ES membranes show that electrospinning increases the ΔH_f values for PEO and PVA by 5% and 19%, respectively. Electrospinning does not affect the T_m for PEO very much, but increases the T_m of PVA by 2°C. Although little has been reported on the molecular order from electrospinning, the higher crystal melting enthalpies of the electrospun membranes indicate that crystallization is induced by electrospinning.

The melting peaks of PEO and PVA in the PEO/casein and PVA/casein membranes indicate the existence of the crystalline domains of these polymers in the fibers. The decreasing melting enthalpies of PEO and PVA with increasing casein contents suggests

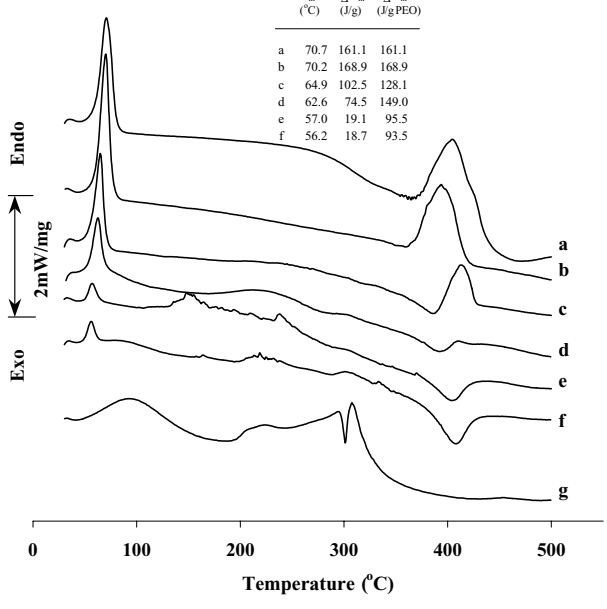

Figure 4 DSC analysis of PEO/casein membranes: (a) 100:0, 5% (cast) (b) 100:0, 5% (c) 80:20, 5% (d) 50:50, 5% (e) 20:80, 5% (f) 20:80, 10%; and casein neat powder (g). The data show the PEO melting point and enthalpy.

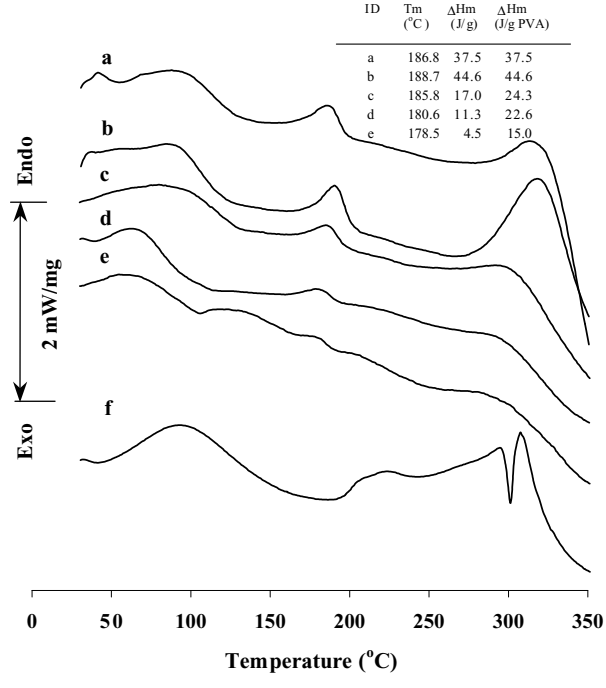

Figure 5 DSC analysis of 10% PVA/casein membranes: (a) 100:0 (cast) (b) 100:0 (c) 70:30 (d) 50:50 (e) 30:70; and casein powder (f). The data show the PVA melting point and enthalpy.

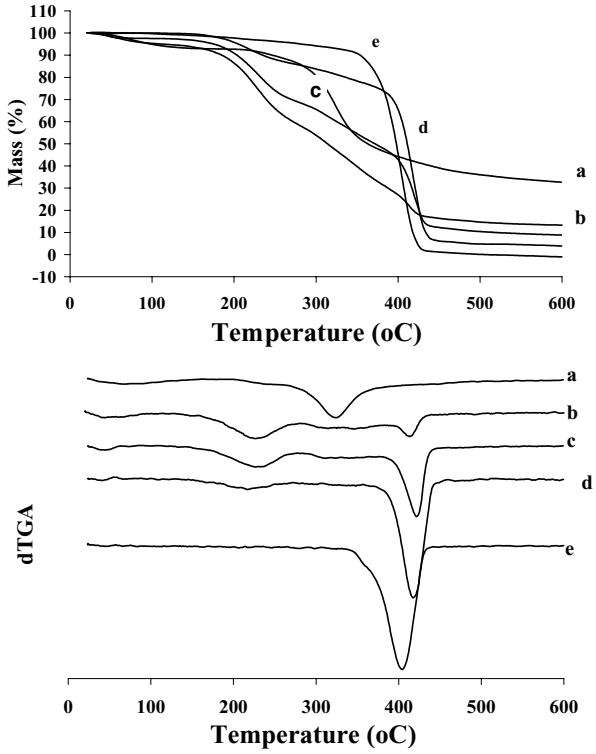

Figure 6 Thermogravimetric (TGA) and their derivative (dTGA) analysis of (a) casein neat powder, and PEO/casein ES membrane (Table Ia): (b) 20:80, (c) 50:50, (d) 80:20, and (e) 100:0.

that the sizes of these crystalline domains decrease with their decreasing contents. The influence of casein on the thermal properties of PEO and PVA also gives evidence to strong interaction between casein and PEO or between casein and PVA. These thermal analyses together with earlier SEM observations and solubility test are consistent with the explanation that both PEO and PVA are highly compatible with casein leading to a well mixed and hydrogen bonded structure with varying levels of phase separation.

The TGA of casein shows a moisture loss, followed by decomposition beginning ∼200°C and continuing over a wide range of temperatures, leaving 30% residue at 600°C (Fig. 6). PEO, which is more thermally stable, losses mass at 350°C and leaves nearly no residue at 600°C. All three electrospun PEO/casein membranes exhibit two-stage decompositions that can be more clearly distinguished by the derivatives of their TGA curves. These non-overlapping derivatives show that casein in PEO/casein decomposed at lower temperatures and the PEO component decomposed at a higher temperature than their pure counterparts. These opposing effects suggest that, in PEO/casein, the structures of these two polymers are affected by the presence of the other in different manner. The casein in PEO/casein membranes is less thermally stable than casein alone, suggesting the casein domains formed in presence of PEO is not as strongly bonded. The inter-molecular and intra-molecular hydrogen bonding capacity of casein is disrupted by its hydrogen bonding with PEO, reducing the size and/or perfection of phase domains and making it more susceptible to heat. PEO molecules alone do not have much inter-molecular and intra-molecular bonding capacity, however, when hydrogen bonded with another polymer such as casein, its thermal stability is enhanced.

3.3. Enzyme-carrying ES membranes

The knowledge gain from electrospinning casein was used to prepare enzyme-carrying ES membranes by replacing casein with lipase. Since the structures and solubility of casein and lipase are different, variations in polymer compositions are expected and made to accomplish electrospinning. The lipase-containing solutions can be electrospun into fine fibrous membranes which show similar morphologies as casein-containing ES membranes (Table II). Lipase was electrospun with PVA at a 20% loading level or with both PEO and casein at 20% to 30% loading levels.

In the enzyme-catalyzed hydrolysis of olive oil, the liberated ─COOH increases linearly with time between 2 and 10 hrs, and remain unchanged after 10 hrs (Fig. 7). The PVA/lipase membrane exhibited higher catalytic activities than the PEO/casein/lipase membrane.

The lipase in PVA/lipase ES membrane is 6 times more active than that in the cast membrane from the same solution (Fig. 8). It is expected that electrospun fibrous membranes serve as better enzyme-carrying substrates because of their higher surface areas and porous structure. If the assay is conducted using ES membrane attached on a solid, i.e., aluminum foil, the activity is lower than that of the detached membrane, but still higher than that of the solvent cast film. The lipase membrane in second round of assay can keep 21% of its original activity. It should be noted there is 5 days of storage time between the two cycles of assays.

TABLE II Electrospinning of lipase containing solutions (10% total concentration)

PEO:casein:lipase (w:w:w)	Spinning observation	Product
	(a)	
40:40:20	Continuous, jet radiation	Fine fiber membrane, partially detachable
30:40:30	Continuous, jet radiation	Fine fiber membrane, partially detachable
PVA:lipase (w:w)	Spinning observation	Product
	(b)	
50:50	Jet observable, no collection	–
80:20	Jet observable, collect slowly	Fine fiber, partially detachable

[a] 10% lipase in buffer mixed with 10% PEO in water and 10% casein in 5% aq. triethanolamine.
[b] 10% lipase in buffer mixed with 10% PVA in water.

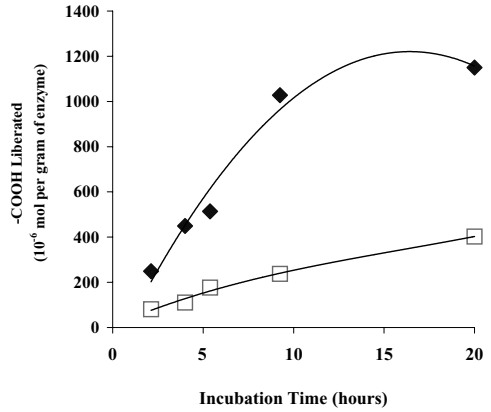

Figure 7 Hydrolysis of oilve oil by lipase incorporated membranes. ♦ PVA:lipase = 80:20 (Table IIb); PEO:casein:lipase = 30:40:30 (Table IIa).

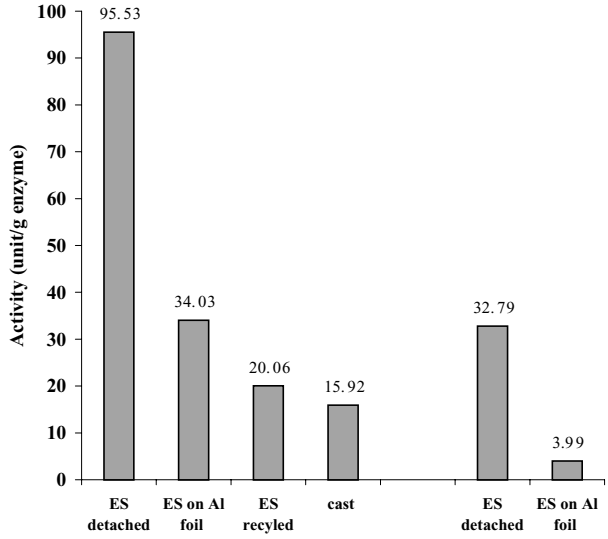

Figure 8 Catalytic activity of enzyme-carrying membranes. left 4: PVA/lipase (Table IIb, 80:20); right 2: PEO/casein/lipase (Table IIa, 30:40:30).

The attachment to a solid foil decreases the total accessible surface area of ES membrane, and makes it less porous. However, this demonstrates the potential of attaching enzyme-carrying membranes to another substrate for versatility of handling, storage, and applications. This may also enhance the environmental stability of the enzymes in the membranes. It is foreseeable that this approach could be further developed by electrospinning the enzyme containing solutions onto different substrates, e.g., polymers, metals, or other materials, to form wide-ranging types of enzyme-containing composites. This could substantially expand the enzyme immobilization approaches. One advantage of solid-supported enzymes is their convenience in repeated usage. After the first cycle of assay, the lipase-carrying membrane could be recycled, cleaned, and dried for further usage. The activity loss during each step including reaction, recycling, cleaning and storage need further quantitative investigation.

These catalytic activities of lipase in ES membranes are about 2 orders of magnitude lower than that of free lipase (9599 unit/g as assayed). This substantial difference may be explained by several reasons. First of all, catalysts in the solid state and/or bond to solids are expected to have much more limited access to the catalyzing substrate (olive oil) than those in the soluble states. Secondly, not all enzymes incorporated in polymer blends reside on the surface nor are exposed to the substrate to participate in the reaction. Furthermore, it is possible that the enzyme structure could be affected by the electrospinning and/or crosslinking reaction. The fact that PVA/lipase membranes exhibit higher catalytic activities than PEO/casein/lipase membranes suggest additional reasons including morphological factors, chemical crosslinking effects, and polymer interaction. More specific studies to address these aspects are being conducted in this laboratory.

4. Conclusions

Electrospinning is a viable and versatile method to produce ultra-thin fibers from natural proteins. Casein, a milk protein, cannot be electrospun by itself due to its strong intermolecular force and 3-D structure. However, by addition of another polymer such as PEO and PVA, polymer solution mixtures of PEO/casein or PVA/casein can be easily and successfully electrosun. The addition of a secondary polymer can dissociate the interconnected polypeptide chain of the protein and therefore reduce its elasticity. By adjusting the polymer concentrations and compositions, a series of fibrous membranes with different casein compositions can be prepared. Fibers in these membranes have diameters generally between 100 and 500 nm.

The PEO/casein and PVA/casein as-spun membranes are instantly soluble in water. Cosslinking the amines on the casein and hydroxyls on PVA and PEO with a diisocyanate renders these protein membranes insoluble in water. Compared with the solvent cast films from the same solutions, the ES fibrous membranes have higher ΔH_f, indicating higher crystallinity. The increased crystallinity may be induced from the electrical stretching force during electrospinning. The T_m and ΔH_f for PEO and PVA in ES membranes decrease with the increasing amounts of casein. This phenomenon suggests that both PEO and PVA are miscible with casein and form varying levels of phase separated structure during electrospinning process.

The lipase enzyme can be incorporated with either PVA or PEO and electrospun. The catalytic activity of lipase in PVA/lipase ES membrane is about 100 fold lower than that of free lipase. However, the lipase in ES membrane is 6 times more active than that in the cast membrane from the same PVA/lipase solution. These results show that electrospun fibrous membranes can serves as excellent enzyme-carrying substrate due to their higher surface area and porous structures. Further work on incorporating enzymes in fibrous structures and optimizing the activities of enzymes in these supports continues in this laboratory.

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