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Coaxial electrospinning and sustained release properties of gelatin-cellulose acetate core-shell ultrafine fibres

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ABSTRACT: Cellulose acetate (CA) ultrafine hollow fibres containing model core protein (i.e., gelatin) were fabricated by coaxial electrospinning. Proper conditions to fabricate the core-shell fibres were investigated with respect to the solution properties (i.e., viscosity, electrical conductivity, surface tension, and interfacial tension) as well as feed rates of core and shell solutions. The core-shell coaxial structures and fibre morphology were examined by transmission electron microscopy and scanning electron microscopy, respectively. In addition, the release characteristics of the coaxial fibrous film were examined in phosphate buffer solution pH 7.4 at 37 °C. The average diameter of the fibres was 392 ± 96 nm, which increased as the core solution feed rate increased. The release mechanism of the gelatin from the fibrous film was anomalous diffusion and exhibited a near zero-order release pattern with a release half-life of $\sim$7.4 days. The fibrous films immersed in the PBS for 20 days were still intact without bursting. The structure of hollow ultrafine fibres of CA (in form of fibrous film) is expected to hold potential for some proteinaceous pharmaceutical/food compounds sustained release in gastro-intestinal tract.

KEYWORDS: encapsulation, protein, drug delivery

INTRODUCTION

Development of novel micro/nanoencapsulation techniques for effective delivery of bioactive compounds has been on the rise to accommodate therapeutic requirements. In general, an encapsulating system is preferably designed to provide zero-order release pattern which is an ideal release characteristic of an encapsulated bioactive compound. The release pattern allows the capsule to release at a constant rate, maintain proper levels of bioactive compound, either in bloodstream or at the target site. However, bursting of micro/nano vesicles often leads to a failure of the ideal release pattern; this is especially the case in gastro-intestinal (GI) tract. Recently, a novel form of continuously long non-woven fibres formed by electrospinning has been reported for its potential to minimize bursting[1]. This is due to the continuously long and high-order aligning structure of molecules as well as to the high porosity of the electrospun fibres. However, bursting also depends on the property of the surrounding medium, polymer types as well as encapsulant. As the electrospinning process progresses, the ultrafine fibres are collected on a collector plate as fibrous film. The electrospun fibrous mats/films have currently gained rapid interest as a potential controlled release candidate in food, pharmaceutical, and medical applications[2–7]. Nonetheless, direct dispersion of proteinaceous substances including enzymes, hormones, and amino acids into an electrospinning solution leads to compromising functional property of these bioactive substances due to the strong solvents commonly used in the preparation of electrospinning solutions.

Coaxial electrospinning provides many benefits over the conventional electrospinning as degradation and metamorphosis of an active encapsulant can be significantly minimized or avoided[8–13]. It has also been noted that burst release of an encapsulant is effectively minimized. Coaxial electrospinning thus exhibits more steady release rate behaviour[8,14–16]. In addition, the shell matrix of the fibre can be tailored to provide additional functional properties[17,18]. Nevertheless, encapsulation of orally administered bioactive compounds using coaxial electrospinning has rarely been attempted. One of the reasons for this limitation involves the choices of both polymers and solvents, which must be edible materials. Although the fabrication of electrospun fibres using all general food-grade materials is feasible[19], the fibres are still single layer in nature. An attempt to modify the single layer to core-shell layer fibres is therefore desirable.

Several proteinaceous bioactive compounds including enzymes, hormones, and living cells, when travelling through the GI tract, are denatured in the stomach due to proteolytic enzyme and concentrated acid. Cellulose acetate (CA) and its derivatives are the naturally materials resistant to proteolytic degradation suitable to be used as biomedical encapsulating materials[20–25]. CA has also been used widely for oral drug coating and encapsulation[26–28]. Besides, CA was also reported for its electrospinnability, even when being used along with a simple edible solvent such as acetic acid[19,29].

In this work, CA was therefore chosen as a shell layer polymer of coaxial electrospun fibre, whilst gelatin was selected as a model core protein. Gelatin was chosen due to its potential to promote bioavailability of some proteinaceous bioactive compounds as well as its bioactivities such as antihypertensive and antithrombotic activities[30]. It is also a 'generally recognized as safe' substance. Besides, random coil structure of gelatin was expected to exhibit higher release rate than that of other larger molecular proteins, leading to shorter required time for release study. In addition, our preliminary study found that gelatin possesses properties that are much preferred over the other basic proteinaceous encapsulants like albumin, which has excessive electrical conductivity that would result in an unstable coaxial electrospinning jet and hence lead to failure to form core-shell structure of coaxial fibres. This work for the first time proposed an idea of using hollow fibres ultrafine structure of CA to encapsulate a model protein compound in order to provide zero-order release kinetics of the protein in the GI-tract. The effects of core and shell solution properties, including interfacial tension, viscosity, and electrical conductivity ratio, as well as feed flow rate on the coaxial fibre formation are reported along with the release mechanisms of the fibrous film.

MATERIALS AND METHODS

Materials

CA powder having molecular weight of 30 kDa (acetyl content = 39.7% and degree of acetyl substitution $\approx$ 2.4) was purchased from Sigma-Aldrich (Switzerland). Gelatin (type B from alkaline bovine hides) powder was purchased from SKW Biosystems Co., Ltd. (Thailand). Protein content of the gelatin powder was 88.5% (dried weight basis) as determined by Kjeldahl analytical method. Poly(ethylene glycol) powder having a molecular weight of 35 kDa was purchased from Merck (Germany). Glacial acetic (AR Grade, Carlo Erba, Italy) was used as a solvent for both CA and gelatin. A non-ionic food-grade surfactant Tween40 (AR Grade, Fluka, Germany) was used as a surfactant for the core solution. One molar NaCl solution prepared from NaCl (AR Grade, Fluka, Germany) and deionized water was used as an electrolyte solution to adjust the electrical conductivity of the electrospinning solution.

Polymer solution preparation

Shell solution of CA was prepared by dissolving the CA powder in 85% acetic acid. The concentration of CA in the solution was 20 wt%. The mixture was stirred at 25 °C for 1 h using a magnetic stirrer. The CA solution was then adjusted by the NaCl solution in order to obtain the desired electrical conductivity. The core solution was prepared by dissolving poly(ethylene glycol) or PEG and gelatin powder in 85% acetic acid; the solid weight blending ratio of gelatin:PEG was 1:9, while the total concentration of the blend solid was 20 wt%. Subsequently, the gelatin-PEG blend solution was homogenized with soybean oil and Tween40 using a homogenizer (IKA labortechnik T25 Basic, Germany) at 13 500 rpm for 5 min to obtain a homogeneous blend core emulsion. The emulsion system of the core played a significant role in promoting a sharp distinct core-shell interface of the coaxial electrospun fibres. In general, an immiscibility of the core and shell solutions can notably prevent/retard phase mixing of the core and shell solutions in conical droplet at the spinneret tip. Percentage of the soybean oil and Tween40 in the blend solution were 2.2 and 1.1 wt%, respectively. The oil blending ratio was determined from the data of a preliminary test (data not shown) as the minimum amount of the oil that yielded a sharp interface between the core and shell solutions left to stand at ambient condition for 24 h.

DETERMINATION OF SOLUTION PROPERTIES

Viscosity

The apparent viscosity of polymer solutions was determined by a modular compact rheometer (Physica MCR 150, Germany) with CC17 probe. Five ml of a sample solution was placed into a cup holder. The measured shear rate was set between 0.01 and 400 s^{-1}. The temperature of the loaded sample was controlled at 25 °C. The reported viscosity was chosen within a linear range of the shear stress-shear rate relationship.

Tensiometry

The surface tension of the core and shell solutions as well as the interfacial tension of the shell to core solutions were measured at 25 °C by Du Noüy ring method using a Dataphysics tensiometer (Dataphysics Model DCT11, Germany). The platinum ring diameter was 18.7 mm. Twenty ml of each tested solution was poured into a clean glass beaker having a diameter of 6.7 cm. The surface tension measurement was carried out as described by Wongsasulak et al[19]. For the interfacial tension measurement, 40 ml of the shell solution was firstly poured into a clean glass beaker and the ring was then lowered into the solution and set zero. Thereafter, 40 ml of the core solution was carefully poured onto the top of the shell solution. The ring was then pulled through the core-shell interface. Interfacial tension (mN/m) is reported as normalized maximum force exerted on the ring passing through the interface. All tests were carried out in triplicate.

Electrical conductivity

Electrical conductivity of the polymer solutions was measured at 25 °C using a digital conductivity meter (Schott Model CG855, Germany).

Coaxial electrospinning (Co-ES)

Schematic diagram of a coaxial electrospinning apparatus and a coaxial electrospinneret are shown in Fig. 1a and b, respectively. The electrospinning apparatus consists of a coaxial spinneret and a syringe pump (New Era Pump Systems NE1000, Farmingdale, NY) mounted on a wood stand. The feed rate of the shell solution was controlled by a pressurizing device composed of nitrogen gas contained in an air tank and an air pressure regulator (GCE-DruVa GmbH, & Co. FMD502-14MA, Germany). High voltage power supply (Gamma High Voltage ES30P-5W, Ormond beach, FL), operated in a positive DC mode, was used to charge the shell solution, whereas a grounded copper plate was used to collect the electrospun fibres. The setup of the spinneret was shown in Fig. 1b. The inner spinneret consists of an inner 10-ml glass syringe (Popper & sons, NY), which is welded to a stainless steel screwed knob, and a needle with an inner diameter of 0.514 mm and an outer diameter of 0.819 mm. The outer spinneret was a 30-ml stainless steel tube mounted to a needle with an inner diameter of 1.194 mm. The coaxial spinneret was set up by screwed-fixing the inner spinneret to the outer spinneret. An o-ring Teflon slab was placed on the surface of the shell solution to stabilize the pressure of the shell solution contained in the outer

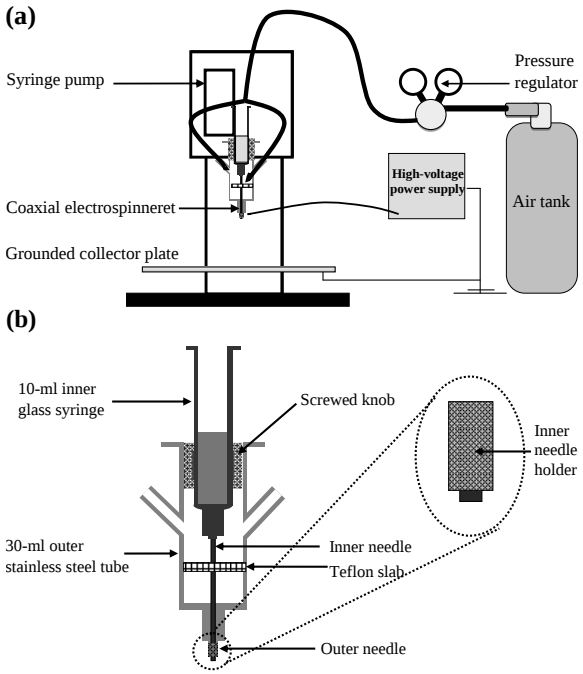

Fig. 1 Schematic diagrams of (a) coaxial electrospinning apparatus and (b) coaxial electrospinneret.

chamber. The annular gap between the inner and outer needles is 0.188 mm. The feed rate of the inner and outer solutions were controlled by a syringe pump (0.18–0.42 ml/h) and the regulation of air pressure (∼0.2 bar), respectively. All experiments were carried out at 25 ± 1 °C and a relative humidity of 50 ± 5 %.

CHARACTERIZATION OF ELECTROSPUN FIBROUS FILMS

Scanning electron microscopy (SEM)

The morphology of the coaxial electrospun fibres was evaluated using a scanning electron microscope (JEOL JSM 6400, MA). The coaxial electrospun films were cut into a 5 cm × 5 cm piece. The sample was then attached to a sample loader and then sputtering coated with gold using a coating condition of 2 kV, 5 mA for 2 min with an argon backfill at 8 Pa. The average diameter of each electrospun sample was determined by image analysis through the use of image analysis software IMAGE J (National Institutes of Health, USA) of 100 fibres.

Transmission electron microscopy (TEM)

The core-shell coaxial structure of the spun fibrous film was investigated with a transmission electron microscope (JEOL JEM-1220, MA) operated at 100 kV with a magnification range of 10 000–100 000 ×. A

Table 1 Apparent viscosity (η_a), consistency index (K), flow behaviour index (n), electrical conductivity (σ), and surface tension (γ) of shell and core solutions.

Sample solution		η_a (Pa s)	K	n	σ (μS/cm)	γ (mN/m)
Shell solution sample†	A	9.41 ± 0.13	13.19 ± 0.40	2.15 ± 0.01	103.3 ± 3.8	31.62 ± 0.40
	B	9.31 ± 0.06	13.27 ± 0.04	2.14 ± 0.00	120.0 ± 1.6	31.69 ± 0.10
	C	8.86 ± 0.11	12.28 ± 0.08	2.15 ± 0.00	132.8 ± 1.0	31.65 ± 0.05
Core solution‡		0.42 ± 0.03	0.08 ± 0.02	1.31 ± 0.06	94.7 ± 2.3	31.66 ± 0.01

† The shell solution sample is added with NaCl at (A) 0.75 wt.%; (B) 0.90 wt.%; and (C) 1.0 wt.% of CA (NaCl was used in form of 1 M NaCl solution).
‡ PEG: GL: OIL: TW40 (wt%) is 9.7: 87.5: 2.1: 1.

sample for TEM examination was prepared by directly electrospinning the fibres onto a TEM copper grid that had been coated with a support carbon-stabilizing Formvar (polyvinyl formal) film without any staining. The sample was then dried in a vacuum oven for 48 h at room temperature prior to subsequent examination via TEM.

Release characteristics

The coaxial gelatin-CA core-shell electrospun fibrous film was cut into a rectangular shape with the dimensions of 2.5 cm × 5.0 cm. The thickness of the fibrous film was measured using a micrometer (Model 102-309, Mitutoyo, Japan) with a precision of ± 1 μm. Each film sample was measured at 10 random positions close to the testing area. Fibrous films with thickness of 110 ± 20 μm were selected for release characteristics investigation. Fourteen pieces of the fibrous films were put into a stainless steel basket. The basket was soaked into 200 ml-phosphate buffer solution (PBS) pH 7.4 containing 0.1 mg/ml of sodium azide; the whole content was contained in a 250 ml-Duran bottle. The release temperature was controlled at 37 ± 1 °C by using a water bath. The solution was stirred using a magnetic stirrer at a constant rotating speed of 100 ± 3 rpm. The release content of gelatin was measured based on the Lowry method as described by Zhou et al[31].

Statistical analysis

Results were subjected to statistical analysis using software of SPSS (Statistical Product and Service Solutions) version 15.0. One-way ANOVA and Duncan's multiple range tests were used to analyse the differences of the investigated data at a significance level of 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Properties of polymer solutions

Based on a preliminary examination of the core and shell solution properties, it was found that the electrical conductivity of the gelatin core emulsion (94.7 ± 2.3 μS/cm) was 3.8 times higher than that of the CA shell solution (25.1 ± 0.3 μS/cm). Such imbalance could result in a failure of the coaxial fibre formation. This is because the higher electrical conductive core jet would be pulled at a higher rate, leading to discontinuity in the core-shell structure formation. In other words, the higher electrical conductivity of the core solution would result in a higher repulsive force, leading to imperfectly wrapping of the core ejected jet by the shell solution. Besides, the higher conductivity of the core material might also induce charge leakage from the shell material, reducing the surface charge density and thereby hampering jetting. Electrical conductivity of the CA shell solution was therefore enhanced by adding NaCl (1 M NaCl solution) at 0.75%, 0.9%, and 1% of CA. Electrical conductivity of the resulting CA solution samples were 103.3 ± 3.8, 120.0 ± 1.6, and 132.8 ± 1.0 μS/cm, respectively (Table 1).

The apparent viscosity (η_a), consistency coefficient (K), flow behaviour index (n), and surface tension of the gelatin core emulsion and the CA shell solutions, as well as the core-shell interfacial tension, are then measured and reported in Table 1. The added salt enhanced the electrical conductivity significantly, with negligible effects on the viscosity or surface tension of the CA shell solution. Core solution composed of PEG, gelatin, oil, and Tween40 exhibited relatively low viscosity and electrical conductivity compared to that of shell solution. The comparative ratios of the viscosity, electrical conductivity as well as interfacial tension of the shell and core solutions were calculated and are shown in Table 2. These properties are generally considered as important keys

Table 2 Ratios of viscosity and electrical conductivity of shell solution to core solution as well as core-shell interfacial tension.

Shell solution sample†	Viscosity ratio (η_s/η_c)‡	Electrical conductivity ratio	Interfacial tension (mN/m)
A	20.9	1.09	21.0 ± 4.9
B	20.7	1.27	20.9 ± 5.6
C	19.7	1.40	21.3 ± 6.3

† The shell solution sample is added with NaCl at (A) 0.75 wt.%; (B) 0.90 wt.%; and (C) 1.0 wt.% of CA (NaCl in 1 M solution).

‡ η_s is viscosity of shell solution, η_c is viscosity of core solution.

to obtain core-shell structure of coaxial electrospun fibre; this is reported and discussed in the following section.

Morphology of coaxial electrospun fibrous film as affected by electrical conductivity and feed rates of core and shell solutions

A successful encapsulation of gelatin emulsion in CA ultrafine fibre kernel was initially indicated by SEM micrograph (Fig. 2a). The pointed arrows indicate the presence of the core components in the fibre kernels. Additional evidence that clearly revealed the core-shell structure is shown by Fig. 2b, which illustrates cross-sectioned fibrous film where gelatin core compound had been removed. However, due to strong electron beam energy hitting directly on the sample surface, the fibre cross-section surface was slightly damaged, leading to slight reduction in the core diameter. Furthermore, the core-shell structure was also characterized by TEM. The TEM micrographs (Fig. 2c) also evidently confirmed that the core-shell structure was formed with a sharp interface between the core and shell layers (as indicated by the core-shell sharp and smooth interface). Addition of soybean oil to obtain the emulsion system of the core also resulted in a higher evaporation rate of solvent from the shell layer of the flying jet, leading to higher viscous drag force acting on the core-shell interface, thereby forming a continuously wrapped core substance in fibre kernels. A similar result was also observed by Yang et al[16].

In general, the ability to form coaxial electrospun fibres with fine core-shell interface depends on both process parameters and solution properties[17,32]. In this work, the effects of solution properties (i.e., electrical conductivity of the core and shell solutions) and process parameters (i.e., feed rates of core and shell solutions) on the morphology of the coaxial electrospun fibres were investigated; the results are shown in Fig. 3 and Fig. 4. All the spun fibres were round shape and their diameter varied depending on feed rate ratio and electrical conductivity ratio between the core and shell solutions.

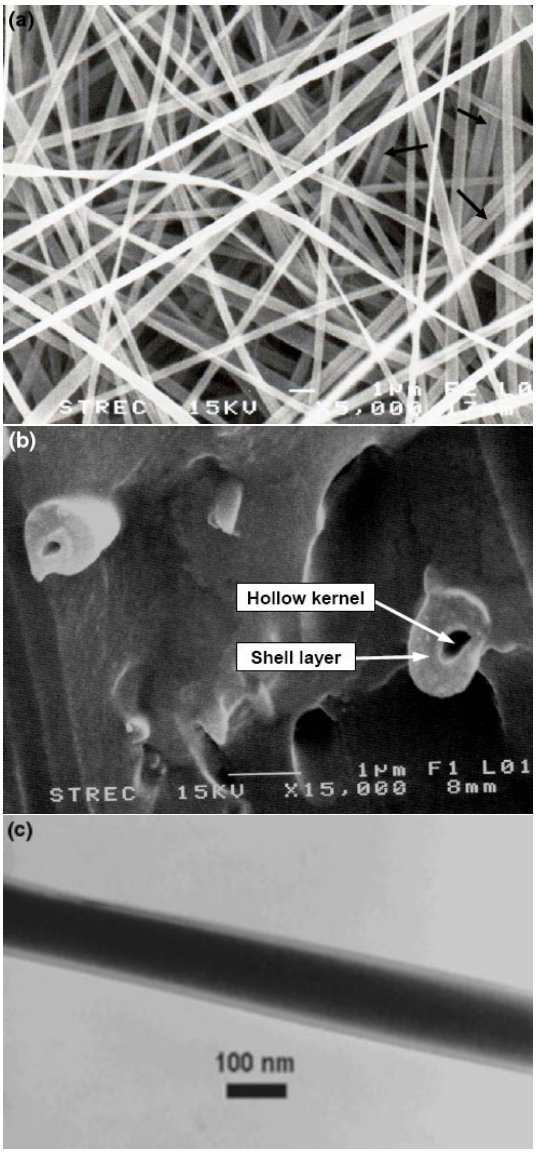

Fig. 2 (a) SEM micrographs of core-shell electrospun fibre composing of CA as shell layer and gelatin blend emulsion as core compound; the arrows indicate coaxial structure of the electrospun fibres, (b) SEM of cross-sectioned hollow fibres after removing core component from the core-shell coaxial fibres, and (c) TEM micrograph of the core-shell coaxial fibres.

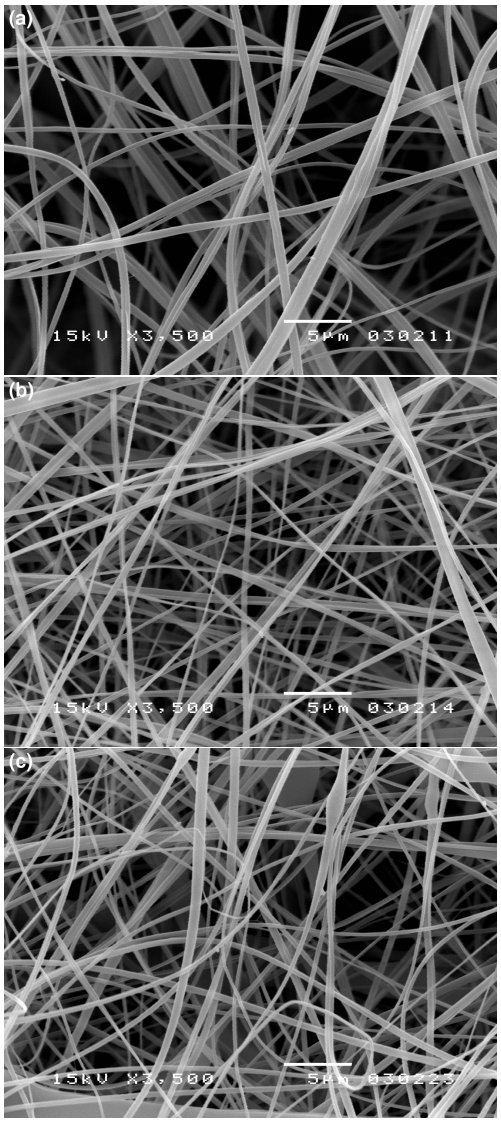

Fig. 3 Effect of ratio of electrical conductivity of shell to core solutions on average diameter of coaxial nanofibre. The electrical conductivity of gelatin core solution was fixed at 94.7 ± 2.3 μS/cm, whereas that of CA shell solution increased from (a) 103.3 ± 3.8 to (b) 120.0 ± 1.6 and (c) 132.8 ± 1.0 μS/cm.

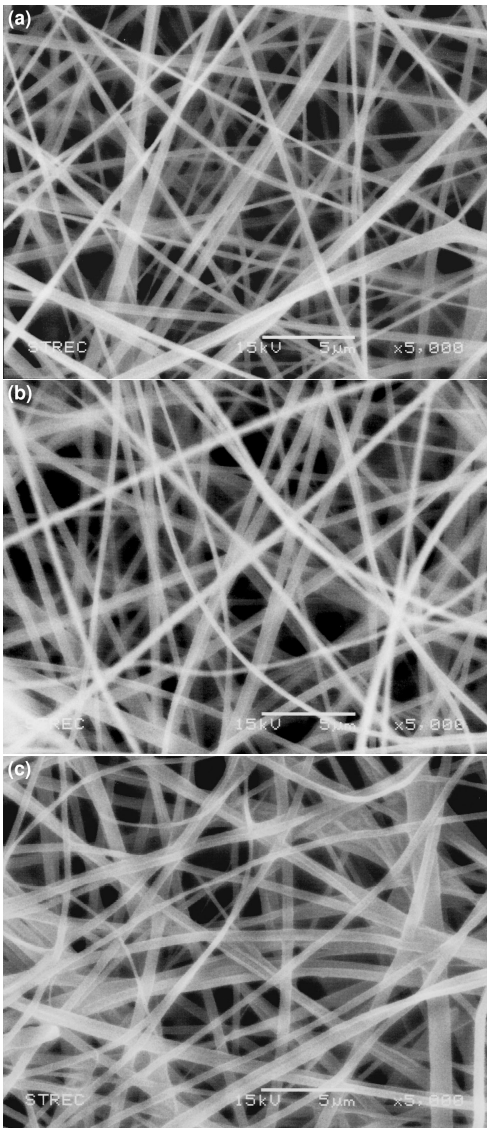

Fig. 4 Scanning electron micrographs of coaxial electrospun fibres at various feed rates of CA solution (a) 1.4 ml/h; (b) 2.1 ml/h; and (c) 2.8 ml/h. Feed rate of core solution was fixed at 0.18 ml/h.

A study on the effect of shell/core electrical conductivity ratio on the fibre diameter was performed by enhancing the electrical conductivity of the CA shell solution from 103.3 ± 3.8 to 120.0 ± 1.6 and 132.8 ± 1.0 μS/cm using 1 M NaCl solution; the value for the core solution was fixed at 94.7 ± 2.3 μS/cm. As the shell/core electrical conductivity ratio increased from 1.09 (Fig. 3a) to 1.27 (Fig. 3b) and 1.40 (Fig. 3c), the average diameter of the coaxial nanofibre significantly reduced from 720 ± 180 to 407 ± 94 and 392 ± 96 nm, respectively ($p < 0.05$). This is probably due to the fact that the increase in the electrical conductivity of the shell solution resulted in higher repulsive force of the stretching jet, thereby yielding smaller fibres. For the effect of the core and shell solution feed rates on the fibre diameters, the results (Fig. 4) showed that when the feed rate of the shell solution increased from 1.40 (Fig. 4a) to 2.10 (Fig. 4b) and 2.80 ml/h (Fig. 4c), at a fixed core solution feed rate of 0.18 ml/h, the diameter

of the resulting fibres insignificantly increased from 376 ± 59 to 400 ± 69 and 407 ± 94 nm, respectively ($p \geqslant 0.05$). In contrast, as shown in Fig. 5, when the core solution feed rate increased from 0.18 (Fig. 5a) to 0.30 (Fig. 5b) and 0.42 ml/h (Fig. 5c) at a fixed shell solution feed rate of 2.80 ml/h, the diameter of the resulting fibres significantly increased from 407 ± 94 to 440 ± 120 and 500 ± 130 nm, respectively, ($p < 0.05$). The results showed that the feed rate of the core solution had a more significant impact on the fibre diameter than that of the shell solution, which implies that the loading content of the encapsulated compound might be increased by directly increasing the feed rate of the encapsulated-core solution.

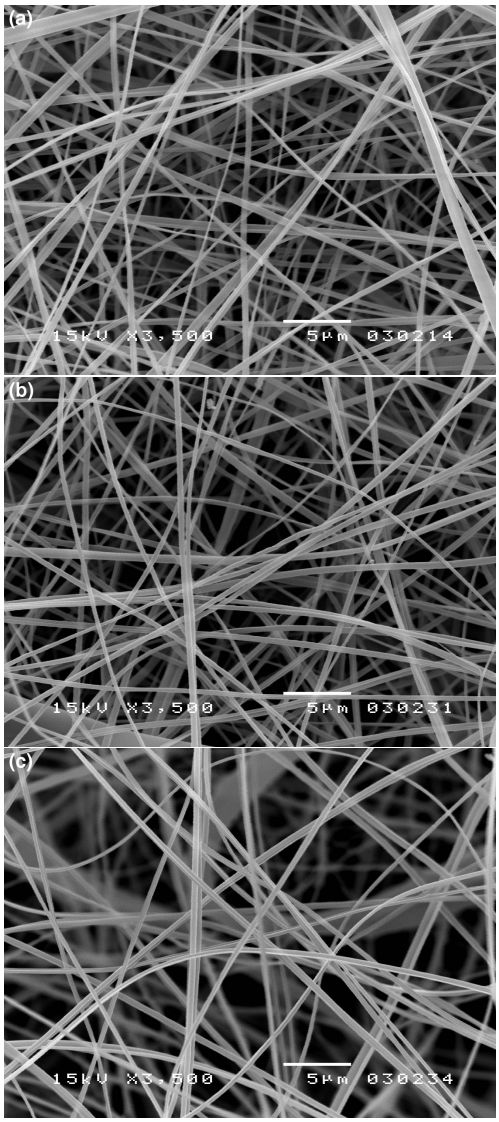

Fig. 5 Scanning electron micrographs of coaxial electrospun fibres at various feed rates of gelatin core solution (a) 0.18 ml/h; (b) 0.30 ml/h and (c) 0.42 ml/h. Feed rate of shell solution was fixed at 2.8 ml/h.

Release characteristics of the gelatin-/CA core-shell electrospun fibre

Shown in Fig. 6 are the release profiles of gelatin core compound from the gelatin-cellulose acetate core-shell electrospun fibrous film in PBS pH 7.2 and at 37 °C, plotted as the gelatin accumulative mass released fraction versus time of release. The profiles showed a linear behaviour with slightly higher release rates at an initial stage (in the first 20 h). The cumulative mass was about 20 wt% at 1 h and then reached 30 wt% at 20 h. The sudden jump to 20% of the gelatin mass detected in the first-stage of the release could be due to some imperfectly encapsulated core gelatin depositing on the fibre surfaces. In addition, it was also possible that some core gelatin leaked from the open-ends of fibres. This was supported by the result of the coaxial fibre mass erosion which exhibited a sudden initial mass loss of ∼16% (data not shown). Hence it is reasonable to subtract the gelatin mass release detected at time t by the 16% detected mass. Filled circles in Fig. 6 then show the normalized data of accumulative mass released fraction $(M_t - M_0)/(M_\infty - M_0)$ versus time, where M_t is the mass released at time t, M_0 is the initial mass suddenly detected at the initial time, i.e., 0.16, and M_∞ is the overall released mass.

In order to determine the release kinetics of the coaxial fibrous film, the normalized cumulative mass release fractions are fitted to a power law model: $(M_t - M_0)/(M_\infty - M_0) = kt^n$, where k is the release rate constant of nth order and n is the release exponent, which can be used to indicate the release pattern, i.e., $n = 0.5$ for square root time kinetics, $n = 1.0$ for zero-order kinetics[33,34]. In general, $n > 0.66$ prevails zero-order release kinetics of the system[34].

In this study, release exponent (n) and release rate constant (k) of gelatin from the coaxial fibrous film were 0.69 and 0.014 $\mathrm{h}^{-0.69}$ ($R^2 \sim 0.99$), respectively. The half-time of release was 178 h or about 7.4 days. The exponent of 0.69 indicated that the mass release mechanism was anomalous diffusion, which revealed that the release rate was mainly governed by the rate of fibre swelling interface moving front[33]. In other words, the release rate of the core protein in PBS was governed by the swelling property of the shell layer; the SEM images of the fibrous film after release indeed showed that the fibre diameter was 1.5-fold larger without bursting or disintegration (Fig. 7). On

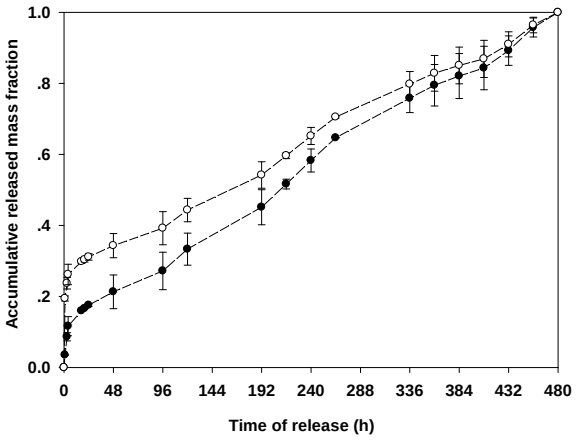

Fig. 6 Release profiles of encapsulated gelatin from gelatin-CA core-shell coaxial electrospun fibrous film in phosphate buffer solution (PBS) pH 7.4 at 37.0 ± 0.5 °C. Open circles: typical cumulative release data; filled circles: normalized cumulative release data.

the other hand, erosion was proved to be a negligible phenomenon in this case (data not shown).

CONCLUSIONS

Since CA has been recognized as a natural polymer having good resistibility to proteolytic enzyme digestion, these CA-electrospun hollow fibres (in form of fibrous film) are thus expected to hold potential for proteinaceous pharmaceutical/food compound sustained release in GI tract. In addition, the study on the effects of process parameters on fibre morphology indicated that the core loading content could be enhanced easily by increasing the feed rate of the core solution during electrospinning. The core-shell fibrous film could, for example, be fabricated and encapsulated in a capsule, which would then be eliminated in the GI tract via digestion to liberate the fibrous film. The encapsulated proteinaceous compound would eventually be released from the fibrous film with zero-order kinetics pattern.

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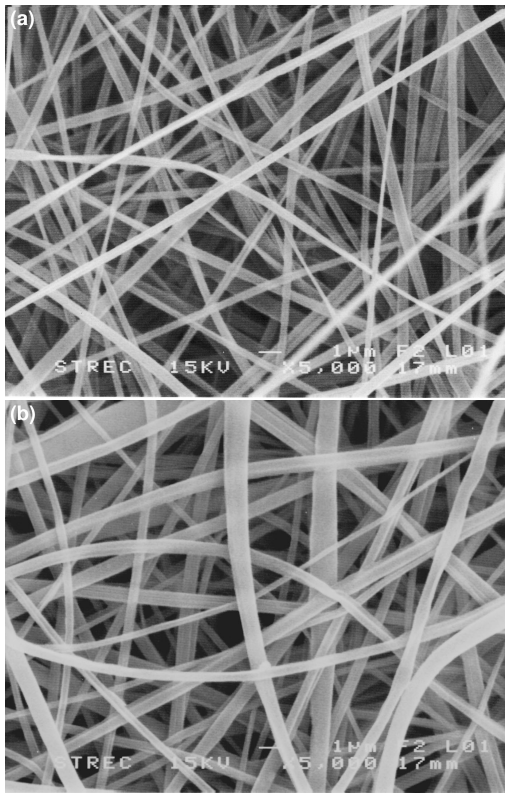

Fig. 7 Scanning electron micrographs of coaxial electrospun fibrous film (a) before and (b) after immersed in PBS pH 7.4 at 37 °C for 20 days.

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