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Biomedical applications of drug loaded protein-based polymer scaffolds and their in-vitro drug release profiles

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Abstract:

Varying amount of drug loaded zein protein nano-scaffolds with different cellulose acetate (CA) and ethyl cellulose (EC) polymer blend ratios were prepared by using single fluid electrospinning. Ketoprofen (KET) was selected as the model drug. Different binary solvent systems were used to electropsun four groups of nano-scaffolds (Group 1-Group 2). These solvent systems includes: 1; acetic acid+ethanol, 2; acetone+ethanol, 3; acetone+N,N-dimethyl acetamide (N,N-DMAc), 4; acetone+N,N-dimethyl formamide (N,N-DMF). Scanning electron microscopy (SEM) was used to investigate the morphologies of four groups of protein-based polymer scaffolds. The average diameter of nanofibers was calculated for each scaffold by using Image J software. Transmission electron microscopy (TEM) suggests smooth surface and even distribution of zein protein particles on nano-scaffolds. In-vitro drug release profiles were used to investigate the release patterns from each fiber mat of four groups. The current research findings reveal that proteins can be used to make scaffolds by mixing them with different polymer blend ratios and different solvent systems can be utilized in making scaffolds. Such scaffolds can be feasibly used as suitable drug delivery systems or for other biomedical applications.

Keywords:

Electrospinning, protein-based scaffolds, polymer blends, zein coated, nano-scaffolds.

Introduction

Nanotechnology is defined as the ability to control and adjust the material at atomic or molecular levels and taking advantage of the distinct properties and phenomena at that scale compared to the bulk properties (Roco, 2011). This innovative technology has several approaches, which have been emerging in a broad range of disciplines with mass applications from electronics to biomedicines (Sozer & Kokini, 2009).

Tremendous research progresses have been made in the past decade towards the design of controlled release drug delivery systems and scaffolds with an

appropriate hierarchical structure to release active proteins and therapeutic agents according to predesigned drug release pattern. Controlled drug delivery systems (DDSs) have been developed in order to avoid the main limitations of conventional preparations. This may lead to significant advances in therapeutic applications of controlled release drug delivery (Son, Kim, & Yoo, 2014).

Protein-based polymer scaffolds have been developed for several biomedical applications including tissue engineering (Mafi, Hindocha, Mafi, & Khan, 2012; Malafaya, Silva, & Reis, 2007) and drug delivery (Sun & Mao, 2012) because of their excellent distinctive properties. Such type of protein-based polymer scaffolds has advantage of several physical similarities of extracellular matrix but also having certain limitations. These limitations can be overcome by their attachment with different chemical groups of drugs, therapeutic agents and other polymers in order to achieve a time controlled degradation rate (Malafaya et al., 2007; Shamshad Ali, 2014). Recently, a broad range of proteins have been utilized for making polymer-based scaffolds including collagen (Ferreira, Gentile, Chiono, & Ciardelli, 2012), gelatin (Awad, Wickham, Leddy, Gimble, & Guilak, 2004), silk fibroin (Hofmann et al., 2006), fibrin (Neidert, Lee, Oegema, & Tranquillo, 2002) and elastin (Machado et al., 2013). In addition to these, zein is another naturally occurring hydrophobic plant protein which can be used for making protein-based scaffolds, drug delivery systems and tissue engineering devices.

Zein is a wonderful biomaterial that can be used as a model tool for drug delivery or tissue engineering. It is one of the most well understood hydrophobic plant protein that is not soluble in water at pH<12 and is a promising biodegradable polymer (Shamshad Ali, 2014). It has been investigated extensively as one of the excellent candidates for environment friendly polymers for several applications (Helan Xu, 2011). Development of technological tools that can be used to convert zein protein into more valuable products, such as high performance biodegradable packaging material or disposable biodegradable microfluidic devices, would increase the utilization of zein as well as help reducing the environmental and economic concerns. Nanotechnology is the advanced technology of interest for altering zein material. By utilizing electrospinning technique, zein nanofiber hybrids have been produced extensively where additional research will be performed which can be beneficial for the fiber market (A. B. Gordon W.

Selling, Alpa Patel, Dennis J. Walls, Christopher Dunlap, Yen Wei, 2007). Zein protein has been successfully blended with several other natural or synthetic bio-polymers such as nylon-6 (A. B. Gordon W. Selling, 2012), cellulose acetate (CA) (Shamshad Ali, 2014), pectin (Liu, Fishman, Hicks, Kende, & Ruthel, 2006), polyvinyl pyrrolidone (Y. N. Jiang, Mo, & Yu, 2012), collagen (Lin, Li, Zhao, Hu, & Zhang, 2012), chitosan (Luo, Zhang, Whent, Yu, & Wang, 2011), single wall carbon nanotubes (SWCNTs) (Dhandayuthapani et al., 2012), polycaprolactone (Nor et al., 2012) and poly (L-lactide) (Chen Yao, 2009) etc.

In this study protein-based polymer scaffolds have been prepared by mixing zein protein with blends of cellulose acetate (CA) and ethyl cellulose (EC). CA is selected because it has been extensively considered for widespread potential applications in the form of electrospun nanofibrous mats (Konwarh, Karak, & Misra, 2013) and EC is selected because it is an inert, hydrophobic polymer and its properties being non-toxic and stability during storage make it appropriate for sustained release matrices (Li-Ya Huang, 2012). The morphological appearance and intensity of making bonds of zein protein with CA and EC polymers have been investigated by using different solvent systems. Ketoprofen (KET) is chosen as a model drug to study how it can be mixed with protein-based scaffolds to decline its transport complications in bio-systems and gastrointestinal side effects by adjusting its amount, time interval and site of release (Yu et al., 2013). The physical appearances of all these four components are shown in Fig. 1. Here, we investigate the effect of different solvent systems on the morphology and release profiles from blends of hydrophobic zein protein with CA and EC polymer blends.

Fig 1. Physical appearances of a: KET, b: Zein, c: CA, d: EC.

1. Experimental

2.1 Materials

Zein was obtained from Aldrich (purity 98%, Milwaukee, WI, USA). CA (white powder; Mw = 100,000 Da) was purchased from Acros (NJ, USA) and used as received. EC (9.0 cPa s) was obtained from Shanghai Yunhong Pharmaceutical Aids and Technology Co., Ltd. (Shanghai, China). Ketoprofen was purchased from Wuhan Fortuna Chemical Co., Ltd. (Hubei, China). Acetic acid, acetone, N,N-dimethylacetamide (N,N-DMAc), N,N-dimethylformamide (N,N-DMF) and anhydrous ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade. Water was distilled twice immediately before use.

2.2 Preparation of spinning solution

Zein protein was mixed with ethanol and its solution was prepared at four different concentrations i.e., 70 (w/v), 65 (w/v), 60 (w/v) and 55 (w/v). While CA and EC solutions were prepared by using different solvent systems at four different ratios i.e., 4:6 (w/w), 5:5 (w/w), 6:4 (w/w) and 8:2 (w/w). The respective solvent systems used for making four groups of drug loaded zein protein polymer blend solutions are given below:

2.2.1 Acetic acid +Ethanol

Zein protein solution was mixed with CA and EC polymer blend solutions with mixture of different binary solvent systems in four groups. In group 1, Zein protein was mixed with CA and EC polymer blend at four different CA:EC ratios (4:6, 5:5, 6:4. 8:2). The binary solvent system of acetic acid+ethanol (8:2, v/v) was used to prepare group 1 solutions. The amount of KET loaded in the CA and EC polymer blends is kept constant in four different protein-polymer blend solutions. The preparation conditions for group 1 polymer blend solutions are shown in Table 1 (Group 1).

2.2.2 Acetone+Ethanol

In group 2, zein protein solution was also mixed with CA and EC polymer blend at four different CA:EC ratios (4:6, 5:5, 6:4. 8:2). The binary solvent system of acetone+ethanol (8:2, v/v) was used to prepare group 2 solutions. The amount of KET loaded in the CA and EC polymer blends was also kept constant in four different protein-polymer blend solutions. The preparation conditions for group 1 polymer blend solutions are also shown in Table 1 (Group 2).

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Table 1. Preparation conditions for different polymer blend solutions and their morphologies

Groups	No.	Ratio CA:EC (w/v)	Zein (w/v)	Drug (mg)	Solvents (8:2, v/v)	Morphology
Group 1	F1	4:6	70	100	Acetic acid +Ethanol	Round, diffused, hollow microspheres
	F2	5:5	65	100	Acetic acid +Ethanol	Round, smooth, globular, hollow microspheres
	F3	6:4	60	100	Acetic acid +Ethanol	Round, hollow, irregular shaped microspheres with very few nanofibers
	F4	8:2	55	100	Acetic acid +Ethanol	Long, cylindrical, smooth nanofibers with spindle shaped beads
Group 2	F5	4:6	70	100	Acetone +Ethanol	Circular shaped solid nanoballs
	F6	5:5	65	100	Acetone +Ethanol	Circular shaped solid nanoballs with very few nanofibers
	F7	6:4	60	100	Acetone +Ethanol	Long, cylindrical, smooth nanofibers with few scattered nanoparticles
	F8	8:2	55	100	Acetone +Ethanol	Long, cylindrical, smooth nanofibers

2.2.3. Acetone+ N,N-DMF

In group 3, zein protein solution was mixed with CA and EC polymer blend at four different CA:EC ratios (4:6, 5:5, 6:4. 8:2). The binary solvent system of acetone+N,N-DMF (8:2, v/v) was used to prepare group 2 solutions. The amount of KET loaded in the CA and EC polymer blends was kept constant in four different protein-polymer blend solutions. The preparation conditions for group 3 polymer blend solutions are also shown in Table 2 (Group 3).

2.2.4. Acetone +N,N-DMAc

In group 4, zein protein solution was also mixed with CA and EC polymer blend at four different CA:EC ratios (4:6, 5:5, 6:4. 8:2). The binary solvent system of acetone+ethanol (8:2, v/v) was used to prepare group 4 solutions. The amount of KET loaded in the CA and EC polymer blends was also kept constant in four different protein-polymer blend solutions. The preparation conditions for group 4 polymer blend solutions are also shown in Table 2 (Group 4).

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Table 2. Preparation conditions for different zein coated polymer blend solutions and their morphologies

Groups	No.	Ratio CA:EC (w/v)	Zein (w/v)	Drug (mg)	Solvents (8:2, v/v)	Morphology
Group 3	F9	4:6	70	100	Acetone + N,N-DMF	Web-like nanofiber mesh
	F10	5:5	65	100	Acetone + N,N-DMF	Long, tilted, cylindrical, smooth nanofibers
	F11	6:4	60	100	Acetone + N,N-DMF	Long, arc shaped, cylindrical, smooth nanofibers
	F12	8:2	55	100	Acetone + N,N-DMF	Long, cylindrical, smooth webbed nanofibers
Group 4	F13	4:6	70	100	Acetone +N,N-DMAc	Long, thin nanofibers with round and spindle shaped many beads
	F14	5:5	65	100	Acetone +N,N-DMAc	Long, thick nanofibers with spindle shaped few beads
	F15	6:4	60	100	Acetone +N,N-DMAc	Long, cylindrical, smooth nanofibers
	F16	8:2	55	100	Acetone +N,N-DMAc	Long, cylindrical, smooth nanofibers

Protein-based polymer blend solutions were covered carefully with paraffin sheets as shown in Fig. 2. Mechanical stirring and persistent heating (50±1.8 h) were applied for at least 12 h to obtain homogeneous co-dissolved spraying solutions. The solutions were degassed with a SK5200H ultrasonator (350W, Shanghai Jinghong Instrument Co., Ltd. Shanghai, China) for 10 min before electro-spinning.

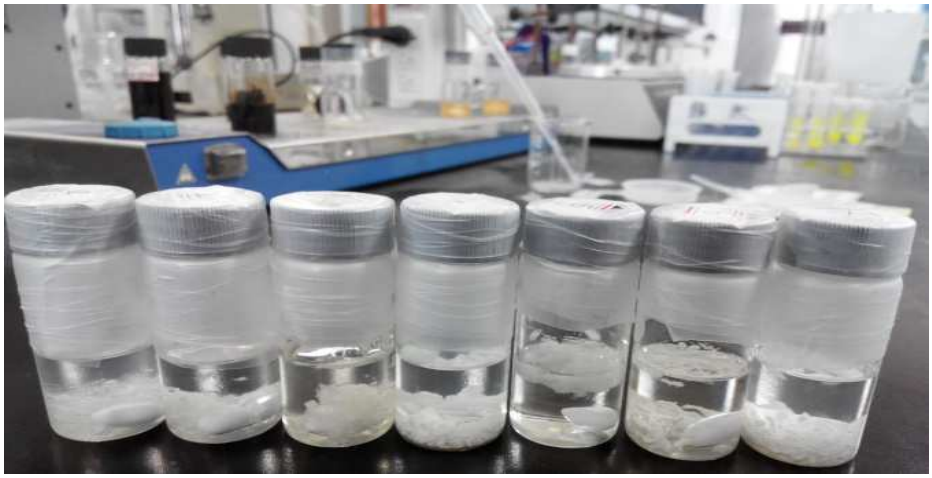

Fig 2. *Preparation of Solutions*

2.3 Electrospinning Process

A high voltage power supply (Shanghai Sute Electrical CO., Ltd.) was used to provide high voltages in the range of 0–60 kV. To avoid air bubbles, electrospinning solutions were carefully loaded in a 5 mL syringe to which a stainless steel capillary metal-hub needle was attached. The inside diameter of the metal needle was 0.5 mm. The positive electrode of the high voltage power supply was connected to the needle tip and the grounded electrode was linked to a metal collector wrapped with aluminum foil. The electrospinning process was carried out under ambient conditions (21 ± 2 ◦C and relative humidity 57 ± 3%). A fixed electrical potential of 15 kV was applied across a fixed distance of 15 cm between the tip and the collector. The feed rate of solutions was controlled at 0.5–1.0 mL/h by means of a single syringe pump (KDS100 Cole-Parmer®, USA). The formed fiber meshes were dried for over 24 h at 40◦C under vacuum (320 Pa) in a DZF-6050 Electric Vacuum Drying Oven (Shanghai Laboratory Instrument Work Co. Ltd., Shanghai, China); this facilitated the removal of residual organic solvent and moisture.

3. Characterizations

3.1 Morphological analysis

The morphology of the nanofibers was assessed using an S-4800 field emission scanning electron microscope (FESEM) (Hitachi, Japan). Prior to the examination, the samples were platinum sputter-coated under a nitrogen atmosphere to render them electrically conductive. Images were recorded at an excitation voltage of 10 kV The average fiber diameter was determined by measuring fiber diameters at over 100 points on the FESEM images using NIH Image J software (National Institutes of Health, MD, USA).

3.2 Transmission electron microscope (TEM)

Transmission electron microscope (TEM) images of the samples were recorded on a JEM 2100 field emission transmission electron microscope (JEOL, Tokyo, Japan). TEM samples of zein coated CA and EC blends were collected by fixing a lacey carbon-coated copper grid on the collector.

3.3 *In-Vitro* release experiments

In-vitro dissolution tests were carried out according to the Chinese Pharmacopoeia (2005). Method II, a paddle method using a RCZ-8A dissolution apparatus (Tianjin University Radio Factory, China) was used. The dissolution medium consisted of 200 mL phosphate buffered saline (PBS) (pH 7.4). The

temperature was maintained at 37°C±1 and the number of paddle rotations was adjusted to 50 rpm. Aliquots of 5 mL were taken at specific intervals and an equal amount of fresh dissolution medium was added to maintain a constant volume of the dissolution medium. The sample solutions were then analyzed at 260 nm by a UV spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China). All the measurements were carried out in triplicate.

4. Results and discussion

4.1 Selection of suitable solvent system for electrospinning of drug loaded zein coated CA+EC polymer blends

Four groups of KET loaded, zein coated CA and EC polymer blend scaffolds were prepared and their respective morphologies were analyzed by SEM. Zein protein was mixed in these four groups at different concentrations in order to determine the optimum concentration for getting bead free nanofibers. These four concentrations were 70 (w/v), 65 (w/v), 60 (w/v), 55 (w/v). In group 1, a binary solvent system of acetic acid+ethanol was used to prepare zein-based scaffolds. The amount of KET was kept constant at 100mg in the four samples. Four ratios of CA:EC polymers were considered (i.e., 4:6, 5:5, 6:4, 8:2) (Table 1) Such type of CA and EC blends have been successfully prepared and investigated in our previous study (Syeda Um-i-Zahra, 2014). The representative SEM images of all the four nano-scaffolds are shown in Fig. 3(a-d). The SEM image of F1 scaffolds were round shaped, hollow and diffused microspheres (Fig. 3a). Zein protein particles were seemed to be scattered on the surface of hollow microspheres. Similar hollow and solid nanoparticles have also been successfully prepared and studied for drug loading and release previously (Helan Xu, 2011). The SEM image for F2 scaffold also showing round, smooth and hollow microspheres but they are also globular in nature. Also zein particles can be seen clearly embedded in the polymer matrix. Some large and some small globules were found (Fig. 3b). SEM image for F3 scaffold display round, hollow and irregular shaped microspheres with few nanofibers (Fig. 3c). Similar zein nanospheres were also prepared in a previous report for the delivery of DNA by the use of simple solvent system and not very high temperatures (H. Jiang, Zhao, & Zhu, 2007). Zein nano and microspheres have been simultaneously used for delivery of ivermectin in a canine model orally (Gong et al., 2011) and also for desmopressin delivery (DiBiase & Morrel, 1997). Contrary to these

nano-spheres, the SEM image of F4 depicts dissimilar results. The F4 scaffold is showing long, cylindrical and smooth nanofibers with spindle shaped beads embedded in the polymer matrix (Fig. 3d). Similar beaded nanofibers were also observed when zein protein was electrospun with incorporation of cyclodextrins (Kayaci & Uyar, 2012). The average recorded diameter of KET loaded, zein coated CA and EC polymer blend scaffolds for group 1 were: F1=1030.08±42nm, F2=957.7±39nm, F3=770.3±24nm, F4=717.4±24nm (Fig. 4a-d). It was observed that as the ratio of CA:EC polymer was increased from 4:6 to 8:2 and zein protein was decreased from 70 to 55, the average diameter of nano-scaffolds decreases.

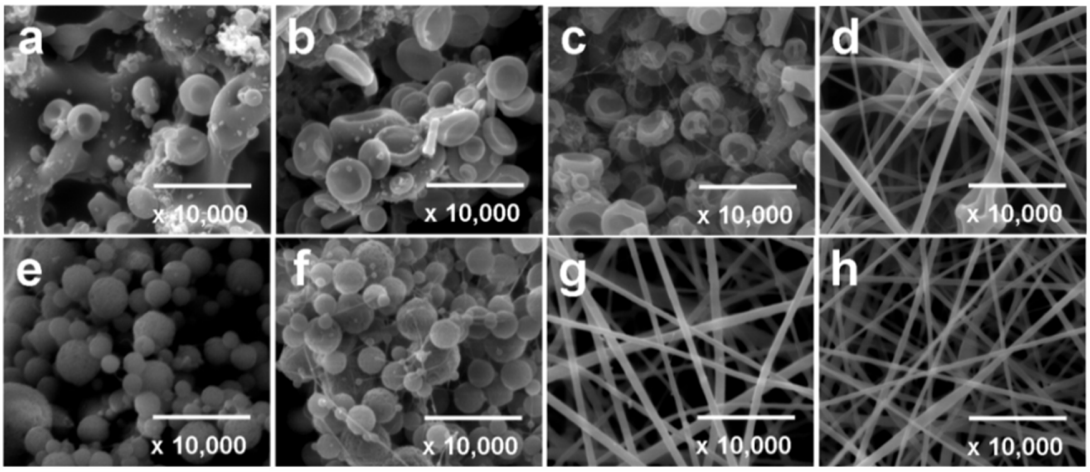

Fig 3. *SEM Images of KET-loaded and zein-coated, CA+EC nanostructures at x 10,000 magnification: a; F1, b; F2, c; F3, d; F4, e;F5, f;F6, g;F7, h;F8.*

In group 2, the binary solvent system utilized was acetone+ethanol to prepare zein-based scaffolds. Similar to group 1, also in group 2, four ratios of CA:EC polymers were considered (i.e., 4:6, 5:5, 6:4, 8:2) (Table 1). The amount of KET was also kept constant at 100mg in group 2. The representative SEM images of all the four nano-scaffolds are shown in Fig. 3(e-h). The SEM image of F5 scaffold was circular in shape with solid nanoballs (Fig. 3e). Zein protein particles were distributed evenly in F5 scaffolds. The SEM image for F6 scaffold shows

circular shaped solid nanoballs with very few nanofibers (very thin) were formed. Zein particles can be seen clearly dispersed in the polymer matrix. Some large and some small nanoballs were appeared (Fig. 3f). When zein protein was mixed with CA and EC polymer blend in the solvent system of acetone+ethanol at lower concentrations, nanofibers were formed as compared to nanoballs. The SEM image for F7 scaffold displays long, cylindrical and smooth nanofibers with few scattered nanoparticles (Fig. 3g). Contrary to F5 and F6 nano-scaffolds, the SEM image of F8 depicts dissimilar results. The F8 nano-scaffold is showing long, cylindrical and smooth nanofibers without any beads or nanoballs (Fig. 3h). The average recorded diameter of KET loaded, zein coated CA and EC polymer blend scaffolds for group 1 were: F5=1150±50nm, F6=937±37nm, F7=788.3±28nm, F8=592±23nm (Fig. 4e-h). It was observed that as the ratio of CA:EC polymer was increased from 4:6 to 8:2 and zein protein was decreased from 70 to 55, the average diameter of nano-scaffolds also decreases.

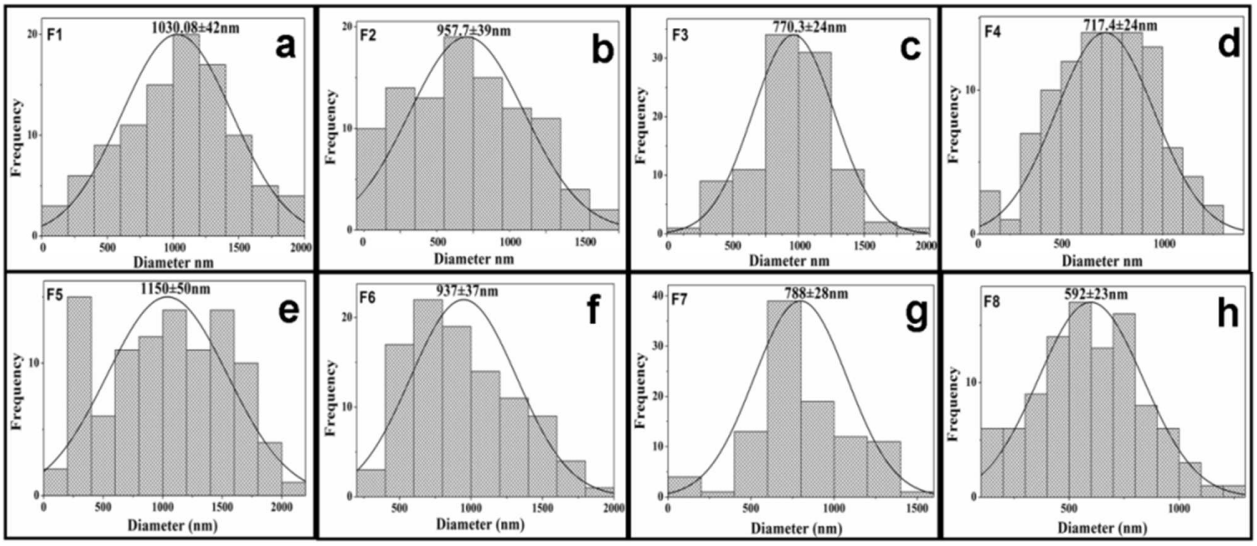

Fig 4. *Graphs showing KET-loaded and zein-coated, CA+EC nanostructures diameter distribution measured from each SEM image: a; F1, b; F2, c; F3, d; F4, e; F5, f;F6, g;F7, h;F8.*

The binary solvent system employed for fabrication of group 3 zein-based protein scaffolds was acetone+N,N-DMAc. A solvent mixture of acetone + N,N-dimethyl acetamide (DMAc) with a

volume ratio of 2:1 and 1:2 (Liu H, 2007; Son WK, 2004; Yan J, 2012) and a solvent mixture of acetone + N,N-dimethyl acetamide (DMAc) + anhydrous ethanol has also been studied previously for the successful fabrication of CA nanofibers. It has also been found that the solvent mixture of acetone + N,N-dimethyl acetamide (DMAc) proves to be the most reliable solvent system for best fabrication of CA-based nanofibers.

Four ratios of CA:EC polymers were also considered (i.e., 4:6, 5:5, 6:4, 8:2) for group 3 nano-scaffolds similarly to group 1 and 2 (Table 2). The amount of KET was also kept constant at 100mg in group 3. The representative SEM images of all the four nano-scaffolds are shown in Fig. 5(a-d). The SEM image of F9 scaffold was showing a web-like nanofiber mesh (Fig. 5a) While the SEM image for F10 scaffold shows long, tilted, cylindrical and smooth nanofibers (Fig. 5b). Some thick and some thin nanofibers were seemed. When zein protein was mixed with CA and EC polymer blend in the solvent system of acetone+ N,N-DMAc at a concentration of 60 (w/v), long, arc shaped, cylindrical and smooth nanofibers were formed. The SEM image for F11 scaffold is shown in Fig. 5c. The nanofibers in F12 nano-scaffold were long, cylindrical, smooth and webbed (Fig. 5d). The average recorded diameter of KET loaded, zein coated CA and EC polymer blend scaffolds for group 3 were: F9=1217±27nm, F10=1113±28nm, F11=849.3±24nm, F12=758±38nm (Fig. 6a-d). It was observed that as the ratio of CA:EC polymer was increased from 4:6 to 8:2 and zein protein was decreased from 70 to 55, the average diameter of nano-scaffolds decreases again.

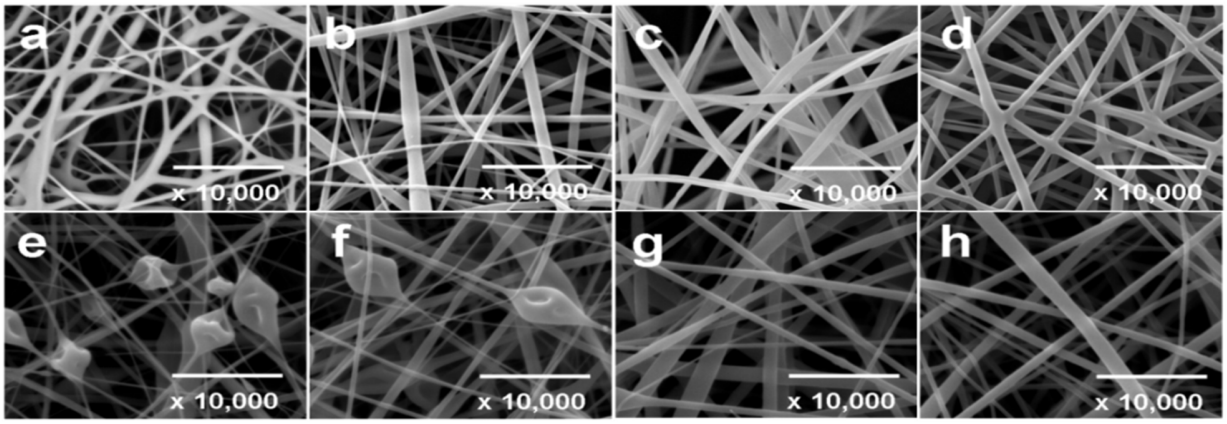

Fig 5. *SEM Images of KET-loaded and zein-coated, CA+EC nanostructures at x 10,000 magnification: a; F9, b; F10, c; F11, d; F12, e; F13, f;F14, g;F15, h;F16.*

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For group 4, the binary solvent system used for fabrication of zein coated polymer scaffolds was acetone+N,N-DMF. The reason for choosing DMF is to get the more thinner and uniform nanofibers having small average diameters (H. Jiang et al., 2007). Following group 1, 2 and 3, again four ratios of CA:EC polymers were prepared (i.e., 4:6, 5:5, 6:4, 8:2) for group 4 nano-scaffolds (Table 2). The amount of KET was also kept constant at 100mg in group 4. The representative SEM images of all the four nano-scaffolds are shown in Fig. 5(e-h). The SEM image of F13 scaffold was showing nanofibers with long and thin nanofibers with round and spindle shaped many beads (Fig. 5e) While the SEM image for F14 scaffold shows long and thick nanofibers with spindle shaped few beads (Fig. 5f). When zein protein was mixed with CA and EC polymer blend in the solvent system of acetone+ N,N-DMF at a concentration of 60 (w/v), long, cylindrical and smooth nanofibers were formed. The SEM image for F15 scaffold is shown in Fig. 5g. The nanofibers for F16 nano-scaffold were long, cylindrical and smooth nanofibers (Fig. 5h). The average recorded diameter of KET loaded, zein coated CA and EC polymer blend scaffolds for group 4 were: F13=927.7±28nm, F14=852.5±23nm, F15=786.2±38nm, F16=757.02±29nm (Fig. 6e-h). It was observed that as the ratio of CA:EC polymer was increased from 4:6 to 8:2 and zein protein was decreased from 70 to 55, the average diameter of nano-scaffolds decreases again. Zein is an amphiphilic prolamine protein which has polar and non-polar functional groups. Zein has thought to improve the physical characteristics of polymer blends and also halo them to become miscible (Nor et al., 2012).

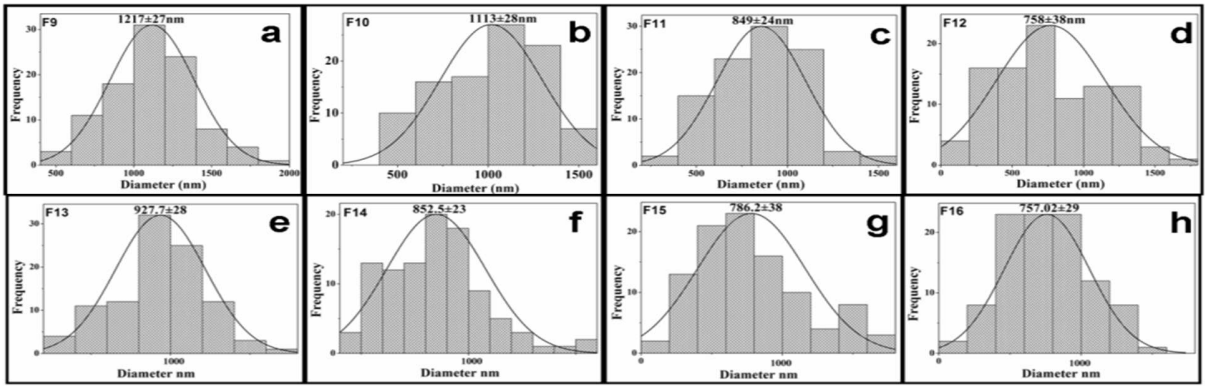

Fig 6. *Graphs showing KET-loaded and zein-coated, CA+EC nanostructures diameter distribution measured from each SEM image: a; F9, b; F10, c; F11, d; F12, e; F13, f;F14, g;F15, h;F16.*

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4.2 Transmission electron microscopy (TEM)

The TEM was conducted to observe the protein-based polymer scaffolds i.e., zein coated CA+EC polymer blends. TEM observations show the homogenous nature of smooth nanofibers. Fig. 7a is showing the TEM images from group 3 nano-scaffolds and Fig. 7b is showing the TEM images from group 4 nano-scaffolds. It is predicted that nanofibers from group 3 and 4 nano-scaffolds have uniform structures with zein particles distributed on it.

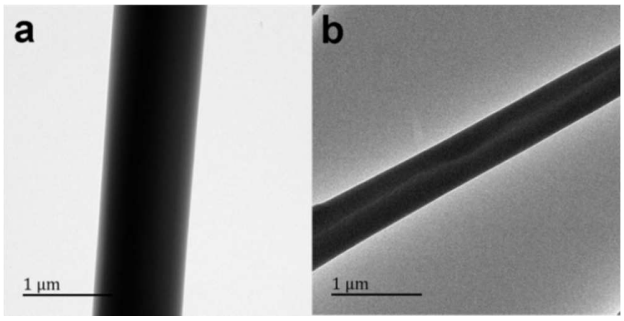

Fig 7. *TEM images of Zein-coated KET-loaded CA+EC polymer blend: a; Group 1 nano-scaffolds, b; Group 2 nano-scaffolds (The scale bars represent 1um).*

4.3 In vitro drug release profiles

KET has a UV absorbance peak at 260 nm. Hence, the amount of KET released from the fibers was determined by UV spectroscopy using a pre-determined calibration curve C = 15.27A–0.0034 (R = 0.9996) where C is the concentration of KET (g/mL^{-1}) and A is the solution absorbance at 260 nm (linear range: 2 to 20 g/mL^{-1}).

As an investigation for drug delivery application of protein-based polymer scaffolds, the zein coated CA + EC polymer blend nanostructures were used for in-situ encapsulation of KET as the model drug. Fig. 7a gives the *in-vitro* drug release profiles of zein coated blended nanostructures obtained under different CA+EC weight ratios (Group 1, Table 1) in PBS (pH=7.4). It was apparent that the four kinds of nanostructures released KET over a 24-h period and maximum KET released into the media was obtained after 3-4 hrs. As shown in Fig. 8a, the zein coated blended nanostructures with higher content of CA and lower content of EC as well as lower zein protein, showed more prolonged release of KET at 37°C. In the first 2 h, nanostructures with higher CA content (8:2, w/w) release about 20% of KET, while other polymer blend solutions prepared under low CA content release about 22% (6:4, w/w), 24% (5:5, w/w) and 26% (4:6, w/w) of KET in first 2 h (Fig. 8a). The release rate of drug from solid zein-coated CA:EC

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nano-scaffolds decreased slowly due to slower release of residual drug molecules. Very interestingly, drug release from hollow zein nano-spheres showed a more sustained and controlled drug release behavior than that from solid zein nano-balls. This will be discussed in details in next section.

The drug release profiles of KET for zein coated blended nanostructures of four different ratios of CA and EC polymer blended nanofibers (Group 2, Table 1) are also given in Fig. 8b. For the F5 nanofibrous mat, the drug release was about 22% in first 2 h. As the CA polymer content increase and EC and zein protein content decrease in blended nanostructures drug release went from fast to slow release respectively. F8 shows higher sustained drug release profiles for 24 hours and F6 is showing least sustained release profiles because both polymer contents were added in same ratios. It is considered that EC is a hydrophobic polymer liable for the prolonged release of hydrophilic drug in the polymer matrix. It decelerates the release frequency in the medium. All of the drug-loaded nanofiber mats (F5-F8) showed a gradual increase in the amount of the drugs released with an increasing immersion time (Fig. 8b). A slower drug release profiles were also observed previously for lysozyme from zein microcapsules produced by anti-solvent process (Qixin Zhong, 2009). Similarly, it was also found that drug release from pectin/zein beads depends on enzymatic degradation of pectin (Liu et al., 2006). This was attributed to the fact that the hindrance of zein particles to the pectin interaction. So it was thought previously that, that zein exists in two main types i.e., strongly bound zein and roughly associated zein. The strongly bound zein protein prevents the pre-mature release of merged drugs or therapeutic agents into the biosystems.

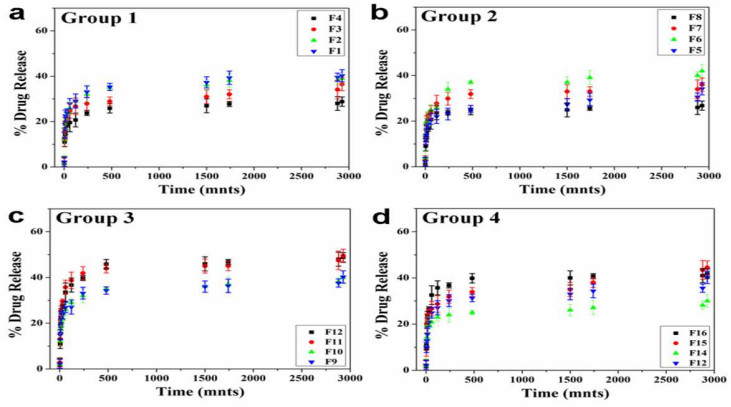

Fig 8. *In-vitro dissolution profiles of KET-loaded, zein-coated CA+EC nanostructures: a; Group 1 (F1-F4), b; Group 2 (F5-F8), c; Group 3 (F9-F12), d; Group 4 (F13-F14) (Data are the mean ± SD of measurements).*

The drug release profiles of KET for zein coated blended nanostructures of four different ratios of CA and EC polymer blend nanofibers (Group 3, Table 2) are given in Fig. 8c. For the F9 and F10 nanofibrous mats, the drug release was about 24-25% in the first 2 h. Zein coated blended nanostructures of F11 and F12 were showing least sustained drug release profiles. As the CA polymer content increase and EC and zein protein content decrease in blended nanostructures drug release went from slow to a little faster release rate respectively. This may happens due to the effect of solvent system for making blend solutions (Fig. 8c) (A. B. Gordon W. Selling, Alpa Patel, Dennis J. Walls, Christopher Dunlap, Yen Wei, 2007).

The drug release profiles of KET for zein coated blended nanostructures of four different ratios of CA and EC polymer blended nanofibers (Group 4, Table 2) are given in Fig. 8d. F14 is showing the highest sustained drug release profile while F16 is showing the least for about 48 h time period. F12 and F15 nanofibrous mats are showing the medium drug release profiles. Here acetone+N,N-DMAc solvent system was used so it may affect the release profile of KET drug (Fig. 8d).

The amount of KET released from the nanostructures seemed to be reliant on the polymer blend ratios. This can be observed from the Fig. 8. Consequently, a percentage of the drug is reserved and locked in the polymer matrix.

To better understand the release mechanisms of the encapsulated KET from the polymer blend nanofibrous mats, the following Peppas equation (Peppas, 1985), which has been successfully utilized to study many other drug delivery systems, was used to fit the *in-vitro* release profiles of the nanofibrous mats:

$$Q = K\mathrm{t}^{n}$$

Where Q is the drug release percentage, t is the release time, k is a constant reflecting the structural and geometric characteristics of fibers and n is the release exponent, which is indicative of the drug release mechanism and the geometry of the device. According to this classification, there are four distinguishable modes of diffusion: (i) the value of n = 0.5 suggests Fickian or Case I transport behavior in which the relaxation coefficient is negligible during transient sorption; (ii) the value of n = 1 refers to a non-Fickian or Case II mode of transport where the morphological changes are abrupt; (iii) if 0.5 <n < 1, the transport

process is anomalous, corresponding to Case III, and the structural relaxation is comparable to diffusion; (iv) a value of n < 0.5 indicates a pseudo-Fickian behavior of diffusion where sorption curves resemble Fickian curves, but the approach to final equilibrium is very slow. By plotting (M_t/M_∞) versus log (t), the n and k values as well as corresponding determination coefficients (R2), were obtained, as listed in Table 3.

Table 3. Release characteristics of encapsulated KET from zein coated blended nanostructures of four different ratios of CA and EC polymer blended nanofibers (Group 1-Group 4)

Groups	No.	Drug loading amount (mg/ml)	Temperature (°C)	n	k	R^2
Group 1	F1	100	25	0.157	0.855	0.945
	F2	100	25	0.255	0.807	0.968
	F3	100	37	0.280	0.881	0.887
	F4	100	37	0.229	1.275	0.896
Group 2	F5	100	25	0.012	1.667	0.865
	F6	100	25	0.041	1.81	0.737
	F7	100	37	0.062	1.605	0.648
	F8	100	37	0.091	1.21	0.699
Group 3	F9	100	25	0.241	0.532	0.673
	F10	100	25	0.244	0.620	0.701
	F11	100	37	0.260	0.626	0.661
	F12	100	37	0.242	0.704	0.647
Group 4	F13	100	25	0.268	0.394	0.625
	F14	100	25	0.218	0.718	0.702
	F15	100	37	0.22	0.762	0.802
	F16	100	37	0.229	0.599	0.725

For the KET loaded, zein coated CA+EC polymer blends nanofibrous mats, group 1 (F1-F4), the n values were found to be in the range of 0.157–0.280 showing the pseudo-Fickian diffusion release mechanism, for group 2 (F5-F8), the n values were found to be in the range of 0.012–0.091 also showing the pseudo-Fickian diffusion release mechanism. Similarly for group 3 (F9-F12) the n values were found to be in the range of 0.241–0.260 showing the pseudo-Fickian diffusion release mechanism and for group 4 (F13-F16), also, the n values were found to be in the range of 0.218–0.268 at 37°C. The release behavior resembles Fickian diffusion behavior, but it is very slow to reach final equilibrium, which may be attributed to the reason that the hydrophilic drug KET being difficult to diffuse out of the hydrophobic zein coated CA+EC polymer blends in PBS at 37°C. In

addition, the k values were found to fluctuate with the increase and decrease of CA and EC polymer contents corresponding to each group.

The regressed results for F1 is $Q=1.14t^{0.157}$, for F2 is $Q=1.378t^{0.255}$, for F3 is $Q=1.512t^{0.280}$, for F4 is $Q=1.81t^{0.229}$, for F5 is $Q=2.9t^{0.012}$, for F6 is $Q=2.4t^{0.041}$, for F7 is $Q=2.3t^{0.062}$, for F8 is $Q=2.3t^{0.091}$, for F9 is $Q=1.702t^{0.244}$, for F10 is $Q=1.871t^{0.260}$, for F11 is $Q=1.859t^{0.244}$, for F12 is $Q=2.022t^{0.242}$, for F13 is $Q=1.484t^{0.268}$, for F14 is $Q=2.052t^{0.218}$, for F15 is $Q=2.142t^{0.22}$, for F16 is $Q=1.821t^{0.229}$.

The present study determines that zein protein can be added into blends of different polymers and by incorporating desirable amount of drug, a desired drug release profile could be achieved by controlling the quantity of zein protein and ratio of polymer blends and binary solvent systems used. Other electrospinning parameters, such as drug concentrations and applied voltage, can be exploited to control the amount of drug release at different phases in making drug-loaded protein-based polymer blended scaffolds. Such kind of protein-based polymer blended scaffolds can also be explored for *in-vivo* experiments and can also be used for several biomedical applications.

5. Conclusions

A single fluid electrospinning was used to prepare KET loaded zein protein nano-scaffolds with different cellulose acetate (CA) and ethyl cellulose (EC) polymer blend ratios. The use of different solvent system displays different morphological features of protein-based scaffolds. Also the average fiber diameters for all the four groups of nano-scaffolds found to decrease with an increase of CA:EC polymer ratios and decrease of zein protein. SEM images demonstrates the round, globular nanospheres and nanoballs, spindle-shaped beads, to long, thin, thick, with/without beads, and web-like nano-scaffolds. The average diameters for group 1 lies between 717.4±24nm to 1030.08±42nm, group 2 lies between 592±23nm to 1150±50nm, group 3 lies between 758±38nm to 1217±27nm and group 4 lies between 757.02±29nm to 927.7±28nm. TEM observations conclude that KET loaded zein protein nano-scaffolds have uniform and homogenous structures. *In-vitro* drug release profiles for group 1 nano-scaffolds are showing that higher zein protein and lower CA:EC polymers showing least sustained release behavior. Drug release profiles for group 2 and 3 nano-scaffolds are showing higher sustained release profiles for lower zein protein and higher CA:EC polymers. While group 4 is showing

contradictory results being least sustained release profiles with lower zein and higher CA:EC polymers. The single electrospinning method used here to generate protein-based polymer scaffolds by mixing them with two different polymer blends can provide a novel way to fabricate other protein-based scaffolds which can widen the ability of this technique to prepare nano-scaffolds with unique structures. This can be very useful step for making protein-based polymer scaffolds for delivery of therapeutic agents to biosystems.

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