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Dexamethasone loaded core–shell SF/PEO nanofibers via green electrospinning reduced endothelial cells inflammatory damage

Weiming Chen[a], Dawei Li[a,e], Ahmed EI-Shanshory[a], Mohamed El-Newehy[c,d,*], Hany A. EI-Hamshary[c,d], Salem S. Al-Deyab[c], Chuanglong He[a,b], Xiumei Mo[a,b,c,*]

[a] College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China
[b] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China
[c] Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[d] Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt
[e] Engineering Research Center of Technical Textiles, Ministry of Education, College of Textiles, Donghua University, Shanghai, China

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ABSTRACT

Silk fibroin (SF)/PEO nanofibers prepared by green electrospinning is safe, non-toxic and environment friendly, it is a potential drug delivery carrier for tissue engineering. In this study, a core–shell nanofibers named as Dex@SF/PEO were obtained by green electrospinning with SF/PEO as the shell and dexamethasone (Dex) in the core. The nanofiber morphology and core–shell structure were studied by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The Dex release behavior from the nanofibers was tested by High Performance liquid (HPLC) method. The protective effect of drug loaded nanofibers mats on Porcine hip artery endothelial cells (PIECs) against LPS-induced inflammatory damage were determined by MTT assay. TEM result showed the distinct core–shell structure of nanofibers. *In vitro* drug release studies demonstrated that dexamethasone can sustain release over 192 h and core–shell nanofibers showed more slow release of Dex compared with the blending electrospinning nanofibers. Anti-inflammatory activity *in vitro* showed that released Dex can reduce the PIECs inflammatory damage and apoptosis which induced by lipopolysaccharide (LPS). Dex@SF/PEO nanofibers are safe and non-toxic because of no harmful organic solvents used in the preparation, it is a promising environment friendly drug carrier for tissue engineering.

1. Introduction

Electrospinning is a highly versatile method to produce continuous nanofibers for drug delivery and tissue engineering. Many biodegradable polymers have been used for nanofiber preparation by electrospinning, such as poly(caprolactone) (PCL), polylactide (PLA) and poly(glycolic acid) (PGA), polyurethane (PU) and so on, however, all these polymers are not soluble in water and only soluble in harmful organic solvents. the toxicity of the organic solvents used could be highly critical and organic solvents are harmful for many reasons such as toxicity and safety issues [1]. To overcome this problem, many water-soluble polymers like silk fibroin (SF), collagen, poly(ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), offer a variety of advantages for electrospinning. "Green electrospinning" is defined that electrospinning of polymers from aqueous solution avoiding harmful organic solvents [2], it is a significant step forwards safe and clean electrospinning.

Silk fibroin has been widely investigated as a biomaterial for biomedical application such as tissue engineering and drug delivery carrier due to its perfect biodegradability, biocompatibility and mechanical properties [3]. In order to increase the viscosity of the solution and obtain a suitable surface tension to generate stable continuous spinning, PEO are commonly added to SF solution, moreover, the PEO phase could be extracted from SF and then can enhance the porosity and surface roughness of nanofibers [4]. Many groups have studied electrospinning of silk fibroin with poly(ethylene oxide) for drug carriers. Li et al. incorporated bone morphogenetic protein-2 (BMP-2) into the SF/PEO scaffolds by directly mixing into the spinning solution and the result showed that BMP-2 enhanced bone formation significantly based on

* Corresponding authors at: State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China; Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail addresses: melnewehy@ksu.edu.sa (M. El-Newehy), xmm@dhu.edu.cn (X. Mo).

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mineralization measures [5]. Fan et al. incorporated L-ascorbic acid 2-phosphate into SF nanofibers by blending electrospinning for skin care application [6]. However, the incorporated drug in those nanofibers are all water soluble drugs, to our knowledge, there was no report that fat-soluble drug been incorporated into SF nanofibers by electrospinning. In our study, fat-soluble drug dexamethasone (Dex) was first incorporated into the core of SF/PEO nanofibers with different Dex contents by emulsion electrospinning. SF/PEO was dissolved in deionized water as shell materials, Dex was dissolved in plant oil and loaded into the core of nanofibers. Not only water-soluble drug incorporate into SF nanofibers, fat-soluble drug can also do so. Here we incorporated Dex into the core of SF/PEO nanofibers and SF/PEO nanofibers protected the drug loaded into the core for controlled release. The release profiles of Dex from core–shell nanofibers showed a slower rate compared with the blending electrospinning nanofibers. This preparation method is regarded as one of green electrospinning since the harmful organic solvents were discarded in the process of electrospinning.

Lipopolysaccharide (LPS) or endotoxin is a highly proinflammatory molecule that elicits a series of endothelial response such as upregulation of cytokines, adhesion molecules, and tissue factor, but beyond that, LPS induce endothelial cell apoptosis in nature [7]. Endothelial cell apoptosis is a bigger threat to life which can lead to disruption of the endothelial barrier with vascular leak, extravasation of plasma proteins, and son on [8]. The treatment of LPS on vascular endothelial cells caused time and concentration dependent reduction in cell viability, The cytotoxic effect of LPS is mediated by NO synthesized, it is mean that LPS can induce endothelial cell damage through excessive production of NO. However, the NO synthesized can be inhibited by dexamethasone [9,10]. Palmer et al. demonstrated that endothelial cells apoptosis induced by LPS (10 μg/mL) can be inhibited by dexamethasone [9]. Meßmer et al. have demonstrated TNF/LPS-mediated apoptosis in glomerular endothelial cells were blocked by dexamethasone, 10 μM dexamethasone blocked roughly 90% of apoptotic cell death in glomerular endothelial cells induced by LPS [11].

Dexamethasone, a steroid anti-inflammatory drug, has shown to reduce the severity of the inflammatory response [12]. Drug-loaded electrospun SF/PEO nanofibers would provide additional advantages for the treatment of tissue defect such as endothelial cells apoptosis induced by LPS. In this study, we demonstrated Dex loaded in the core of nanofibers have a good performance to reduce the Porcine hip artery endothelial cells (PIECs) apoptosis induced by LPS. It is a potential drug carrier for blood tissue engineering or skin tissue engineering.

2. Materials and methods

2.1. Materials

The preparation of the regenerated SF was done as described in our laboratory's previous report [6,13]. PEO (average $M_v \sim 900{,}000$) and lipopolysaccharide (LPS, *E. coli* serotype 055:B5) were purchased from Sigma–Aldrich Trading Co., Ltd. (Shanghai, China). Tween 80 and dexamethasone were purchased from J&K chemical Co., Ltd. (Shanghai, China). 4′,6-Porcine hip artery endothelial cells (PIECs) were supplied by Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). All other chemicals were of analytical grade and commercially available.

2.2. Preparation of emulsions and electrospinning

0.56 g SF and 0.14 g PEO were dissolved completely in 5 mL deionized water as water solution, the concentration of SF/PEO was 14% (w/v); 5 mg and 10 mg Dex was dissolved in 4 mL plants oil and 1 mL Tween 80, respectively, as oil solution, the concentration of Dex was 1 mg/mL and 2 mg/mL (w/v); sequently, 100 μL oil solution was added in 5 mL water solution (1:50, v/v), this was followed by stirring the mixtures at 240 rpm for 12 h to obtain uniform emulsions.

The emulsions were filled into a 2.5 mL plastic syringe with an 18 gauge needle, an aluminum foil was used as receiving plate to collect nanofibers. The distance between the needle and the aluminum foil collector was 15 cm. The syringe was loaded in a syringe pump (789100c, Cole-Parmer Instruments, USA) and dispensed at a rate of 1 mL/h at the applied voltage of 16 kV using a high voltage power supply (BGG6-358, BMEICO, Ltd., China). All the processes of electrospinning were operated at 37 °C with the relative humidity of 20–30. The prepared nanofibrous mats (Dex@SF/PEO) were placed in vacuum to remove the residual solvent. After vacuum-drying, Dex@SF/PEO nanofibers were treated under an atmosphere of 75% (v/v) ethanol vapor (the treatment time ranged from 12 to 24 h). Accordingly, the E1-Dex@SF/PEO (Dex/oil = 1 mg/mL) nanofibers and E2-Dex@SF/PEO (Dex/oil = 2 mg/mL) nanofibers were obtained by emulsion electrospinning.

Dex-SF/PEO nanofibers were also prepared by blending electrospinning. 0.56 g SF and 0.14 g PEO were dissolved completely in 5 mL deionized water to prepare the SF/PEO water solution of 14% (w/v) concentration, then 0.2 mg Dex was dissolved in the SF/PEO solution. The parameter of blending electrospinning was the same as emulsion electrospinning. A series of neat SF/PEO nanofibers without drug were also prepared as controls under the same electrospinning parameter as describe above. Accordingly, Dex-SF/PEO (with Dex) nanofibers and neat SF/PEO (without Dex) nanofibers was obtained by electrospinning.

2.3. Characterization

The surface morphologies of nanofibers were observed by scanning electron microscope (SEM, Hitachi TM-1000, Japan) at an accelerating voltage of 5 kV. Samples were splattered with gold before observed. Visualization software Image-J (National Institutes of Health, USA) was used to measure the diameters of electrospun fibers. Average nanofiber diameters and diameter distributions were determined by measuring at least 100 random fibers from the SEM micrographs. Verification of the core–shell structure was observed by TEM (H-800, Hitachi) at 100 kV, the method was observing the nanofibers which were collected on carbon-coated Cu grids [14].

2.4. In vitro drug release

In the controlled drug release study, two different nanofibers (Dex-SF/PEO and E2-Dex@SF/PEO) were used. Each piece of drug loaded nanofiber mats (about 40 mg) was soaked in a centrifuge tube filled with 2.5 mL of PBS buffer (pH 7.4). The centrifuge tube was incubated in a thermostatted shaker at 37 °C, and the shaking speed was 100 rpm. At appropriate time, 1 mL of the release media was removed from the centrifuge tube and replaced by an equal volume of fresh media. The sample was analyzed using HPLC method to determine the Dex concentration using an injection volume of 20 μL. HPLC analysis was performed using Varian 920-LC series HPLC system (Varian, USA) with a C18 column (4.6 mm × 15 cm) and the released Dex was detected at 242 nm. The mobile phase was acetonitrile:water:phosphoric acid (30:70:0.5%, v/v/v), the flow rate was set at 1 mL/min [15]. The average value was obtained from four parallel samples.

2.5. Effect of LPS on the viability of PIECs

PIECs were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) which contain 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin. The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. PIECs (1×10^4 cells/well) were seeded in 24-well plates which contain 400 μL medium as described above, and incubated overnight at 37 °C for 24 h to allow cells to attach to the plate. Then the medium was exchanged by the DMEM medium with different concentration of LPS (0 μM, 1 μM, 5 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM) to culture cells for 24 h. Then the culture medium was removed and the cultured cells were washed twice with PBS, 40 μL 5 mg/mL MTT solution and 360 μL DMEM were added to each well to continuous culture for 4 h, thereafter, the medium was completely removed and the formazan formed was dissolved in 400 mL of dimethylsulfoxide (DMSO). 100 μL of supernatant was transferred to 96-well microplates and the absorbance were measured at 492 nm with a microplate reader (Multiskan MK3; Thermo Labsystems Co., China). The average value was obtained from six parallel samples.

2.6. Effect of Dex on the cytotoxicity induced in PIECs by LPS

PIECs (1×10^5 cells/well) were seeded in 24-well plates which contain 400 μL DMEM, 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin, and incubated at 37 °C for 24 h to allow cells to attach. Then the medium was removed and exchanged by DMEM, which contain LPS (50 μM) and different concentration of Dex (0, 0.1, 1 μg/mL). Plates were incubated for 24 h at 37 °C. After incubation, the living of cells were observed by viable cell counts (Count star Automated Cell Counter, Shanghai Ruiyu Biotech Co., Ltd). The average value was obtained from three parallel samples.

2.7. Effect of Dex@SF/PEO nanofibers mats on the cytotoxicity induced in PIECs by LPS

The protective effect of E1-Dex@SF/PEO nanofiber mats on PIECs against LPS-induced damage was evaluated by the MTT assay. The same number of PIECs (1×10^4 cells) were seeded on four different mats placed in 24-well plate, which respectively were (+) positive control, (−) negative control, SF/PEO nanofiber mats, E1-Dex@SF/PEO nanofiber mats. The (+) positive control group and (−) negative control group were all glass slide, but the medium was different. For (+) positive control group, cells cultured on 400 μL DMEM which contain 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin, and incubated at 37 °C for 24 h. Then the culture medium was changed by 200 μL DMEM and 200 μL PBS, and cells were incubated at 37 °C for another 24 h. For (−) negative control group, cells cultured on 400 μl DMEM which contain 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin, and incubated at 37 °C for 24 h. Then the culture medium were changed by 200 μL DMEM and 200 μL 100 μM LPS, and cells were incubated at 37 °C for another 24 h. The cell on SF/PEO nanofiber mats and E1-Dex@SF/PEO nanofiber mats were cultured in the same medium condition as (−) negative control group. Cell viability of different groups was determined by MTT assay. Briefly, culture medium was removed and the cultured cells were washed twice with PBS, 40 μL 5 mg/mL MTT solution and 360 μL DMEM were added to each well to continue to culture for 4 h, thereafter, the medium was completely removed and the formazan formed was dissolved in 400 mL of DMSO. 100 μL of supernatant was transferred to 96-well microplate and the absorbance was measured as described above. The average value was obtained from four parallel samples.

The morphologies of PIECs seeded on four groups as described above were studied by SEM and confocal laser scanning microscopy (CLSM). For SEM observation, the cells were washed with PBS and fixed with 4% paraformaldehyde in PBS buffer for 2 h at 4 °C. Then the fixed samples were dehydrated in an ascending series of ethanol for 8 min each and dried in a vacuum. Then the samples were observed by SEM [16]. For the CLSM observation, the cells were washed with PBS and fixed in 4% paraformaldehyde for 2 h and then washed and permeabilized in 0.1% Triton X-100 for 5 min, and then stained with DAPI solution (100 nM) for 5 min. Images were captured using a CLSM [17].

2.8. Statistical analysis

All experiments were conducted at least in triplicate. Statistically significant differences were obtained by comparing data using one-way ANOVA. Data were expressed as mean ± SD and the criteria for statistical significance were $^*p < 0.05$.

3. Results and discussion

3.1. Preparation of nanofibers

The process of Dex@SF/PEO nanofibers preparation is shown in Fig. 1 as described above.

The process for SF/PEO nanofibers by green electrospinning was not easy. SF and PEO can dissolve be in many organic solvents, such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), but the organic solvents should be avoided because of their toxicity, we want to prepare the nanofibers by green electrospinning without using any organic solvent, so we choose water as solvent for electrospinning. The following problem was the evaporation rate of water was slower than the organic solvent which made the SF/PEO nanofibers electrospinning difficult. To overcome this problem, the electrospinning temperature should be controlled at about 37 °C, and the environment humidity should be controlled between 20% and 30%. The solubility of Dex in water was poor, but we found that the solubility of Dex in plant oil was better than in water, 1 mg Dex cannot be dissolved in 1 mL PBS buffer solution completely, however, it can been dissolved in 1 mL plants oil, moreover, the addition of Tween 80 can increase the solubility of Dex in plant oil. Tween 80 was also used as emulsifier for emulsion formation. In the present study, SF/PEO nanofibers with smooth surface could be prepared at the polymer concentration between 12% and 18% (w/v), in this study, the concentration of electrospinning solution was chosen as 14%. 1–20 mg Dex can be dissolved in 5 mL plants oil and Tween 80 (plants oil:Tween 80 = 4:1, v/v) solution. The concentration of Dex was chosen at 1 mg/mL and 2 mg/mL (Dex/(plants oil + Tween 80), w/v) for this study, which were respectively used to prepare E1-Dex@SF/PEO nanofibers and E2-Dex@SF/PEO nanofibers.

3.2. Characterization of nanofibers

The morphology and structure of SF/PEO nanofibers and Dex@SF/PEO nanofibers were analyzed by SEM and TEM. As seen from Fig. 2, the oil phase has little effect on the morphology and diameter of the nanofibers. The average diameter of E1-Dex@SF/PEO nanofibers (511.11 ± 109.03 nm) (Fig. 2b and e) and E2-Dex@SF/PEO nanofibers (528.50 ± 148.00 nm) (Fig. 2c and f) were a little bigger than neat SF/PEO nanofibers (446.25 ± 113.93) (Fig. 2a and d). It can be explained that the addition of plant oil and Tween 80 increased the viscosity of solution which then caused the bigger diameter of fibers during electrospinning [18]. The structure of Dex-SF/PEO nanofiber and E1-Dex@SF/PEO nanofiber can be seen clearly by TEM micrographs as shown in Fig. 3. No core–shell structure was given on Dex-SF/PEO nanofiber (Fig. 3A and C), however, core–shell structure can be seen clearly on E1-Dex@SF/PEO nanofiber (Fig. 3B and D).

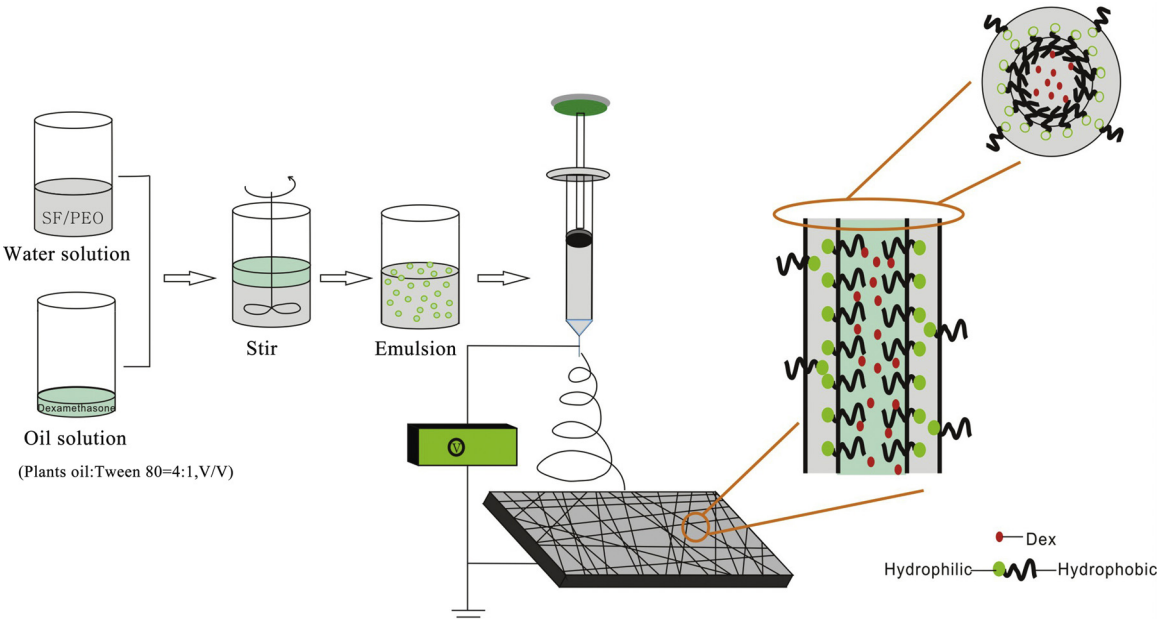

Fig. 1. Schematic illustration of the process for Dex@SF/PEO nanofibers fabrication by emulsion electrospinning.

3.3. Dex release study

Release behaviors of Dex from drug loaded nanofibers was studied and shown in Fig. 4. Two drug loaded nanofiber mats made from blending electrospinning (Dex-SF/EPO nanofiber mats) and emulsion electrospinning (E2-Dex@SF/PEO nanofiber mats) were used, and those two nanofiber mats contained the same theoretical amount of Dex. The results of Dex release from both nanofibers showed that two nanofiber mats all exist initial little burst release in the first few hours, and then the drug released in a relatively steady manner. The burst release of drug from the electrospinning nanofibers at the initial stage was not the result we want and was one of problems for researchers to solve [19]. In a biodegradable system, the mechanism of release of the drug is affected by diffusion as well as degradation of material [20], the drug release was mainly caused by diffusion at the initial stage and degradation of the polymer in later periods [21], so it was easy to understand that the Dex located in the surface of nanofibers easily released from nanofibers to PBS buffer solution at the initial stage. However, there exist difference between blending electrospinning nanofibers and emulsion electrospinning nanofibers on Dex release, the release rate of Dex from Dex-SF/PEO

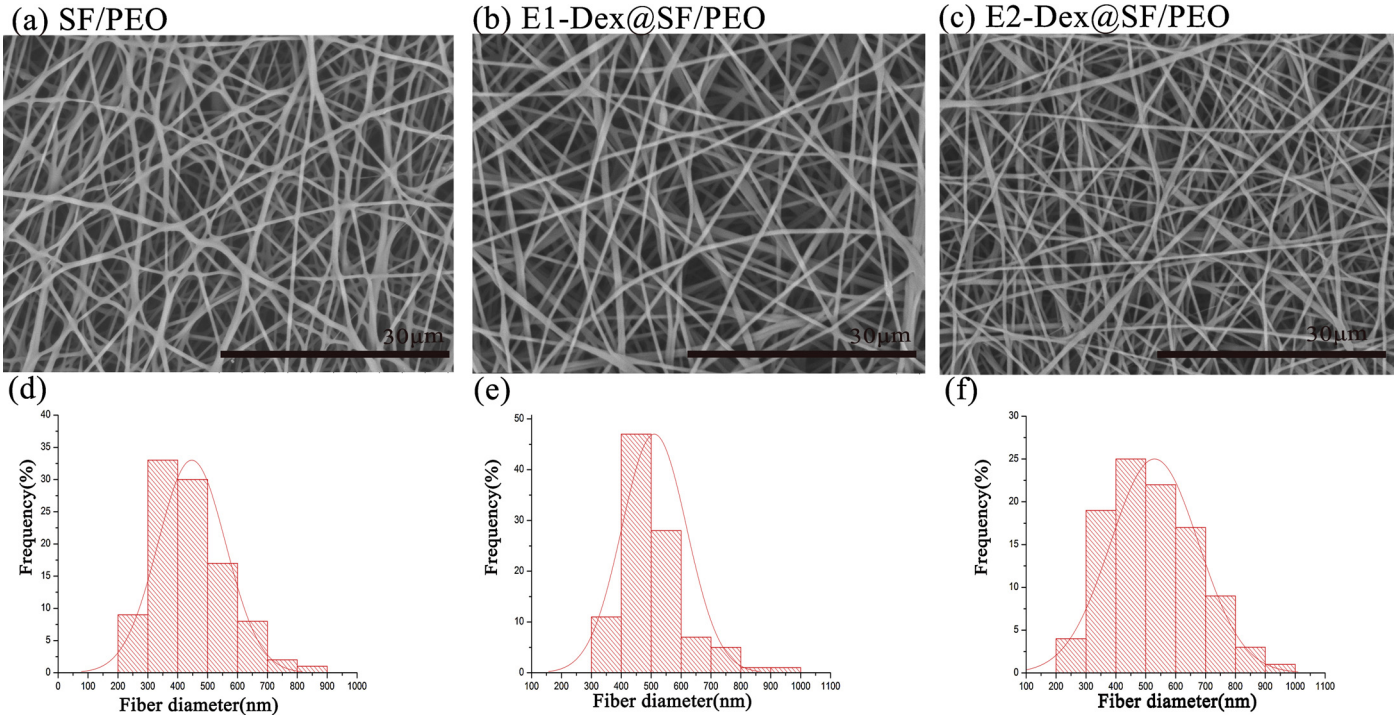

Fig. 2. The morphology and diameter distribution of SF/PEO and Dex@SF/PEO nanofibers. SEM images of (a) neat SF/PEO, (b) E1-Dex@SF/PEO, (c) E2-Dex@SF/PEO, (d–f) the corresponding diameter distributions of a–c.

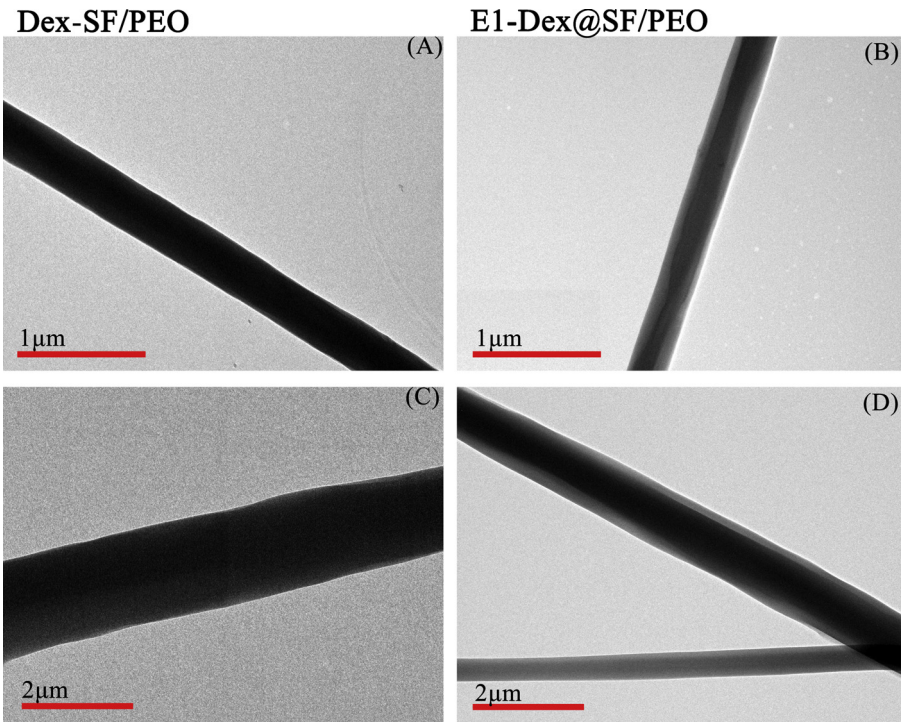

Fig. 3. TEM images of (A, C) Dex-SF/PEO nanofiber, (B, D) E1-Dex@SF/PEO nanofiber.

nanofibers mats showed relatively higher than E2-Dex@SF/PEO nanofibers mats as shown in Fig. 4A, In the first 2 h, the release of Dex from Dex-SF/PEO nanofibers mats and E2-Dex@SF/PEO nanofibers mats respectively were 30% and 21% approximately, and in 24 h, the amount of drug released reached 58% and 42% respectively. Compare the release behavior of Dex from Dex-SF/PEO nanofibers mats and E2-Dex@SF/PEO nanofibers mats as shown in Fig. 4B, we found emulsion electrospinning nanofibers can slow down the release rate of Dex, the possible reason was that the oil phase was loaded into the core of fibers and the water phase was distributed on the surfaces of fibers. During the process of electrospinning, the water may be evaporated quickly during the fiber stretching and solidification, hydrophobic drug such as Dex was expected to be encapsulated in the core of fibers instead of escaping on the fiber surfaces. Many researchers [21–26] have reported that emulsion electrospinning could generate the core–shell structure fibers and incorporate drugs or proteins in the core part. In fact, the Dex distributed in all parts of Dex-SF/PEO nanofibers prepared by blending electrospinning was easy to diffuse through the nanofibers, but most of Dex was loaded in the core of E2-Dex@SF/PEO nanofibers, it need to be defused through the SF/PEO shell for releasing out, which take time and made a slow release.

To our knowledge, there was no report that Dex has been incorporated into core–shell SF/PEO nanofibers by electrospinning and we are the first to report this. The advantages of Dex loaded in the core–shell structure nanofibers prepared by emulsion electrospinning can be described below; (1) the addition of Tween 80 can improve the solubility of drug in the oil solution and increase the drug loaded amount in the nanofibers. (2) Core–shell structure can slow the release of drug from the nanofibers. The ultimate proportion of released Dex in 192 h from Dex-SF/PEO nanofibers was 67.153% and from E2-Dex@SF/PEO nanofibers was 56.684% in Fig. 4B.

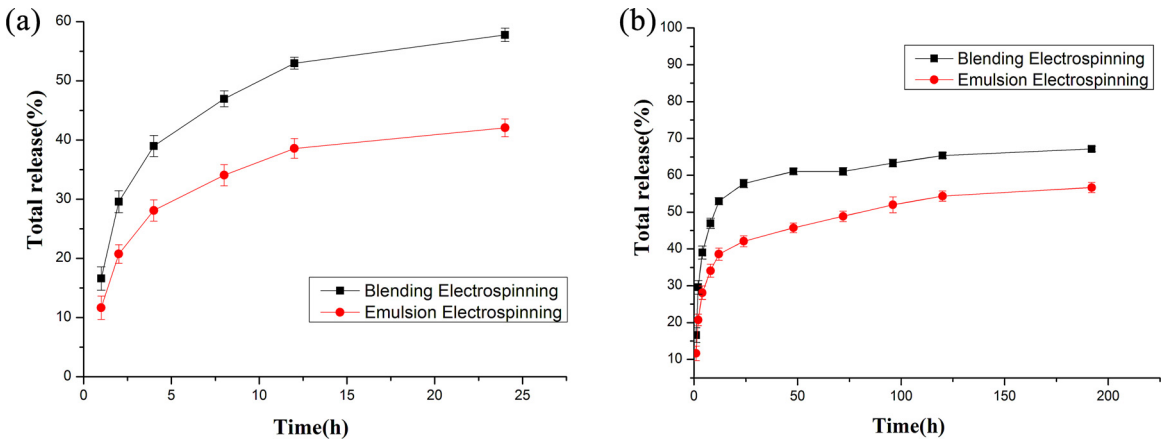

Fig. 4. The cumulative Dex release profiles from two different nanofibrous mats (black line was Dex-SF/PEO nanofibers mats prepared by blending electrospinning, red line was E2-Dex@SF/PEO nanofibers mats prepared by emulsion electrospinning) for 24 h (a) and 192 h (b).

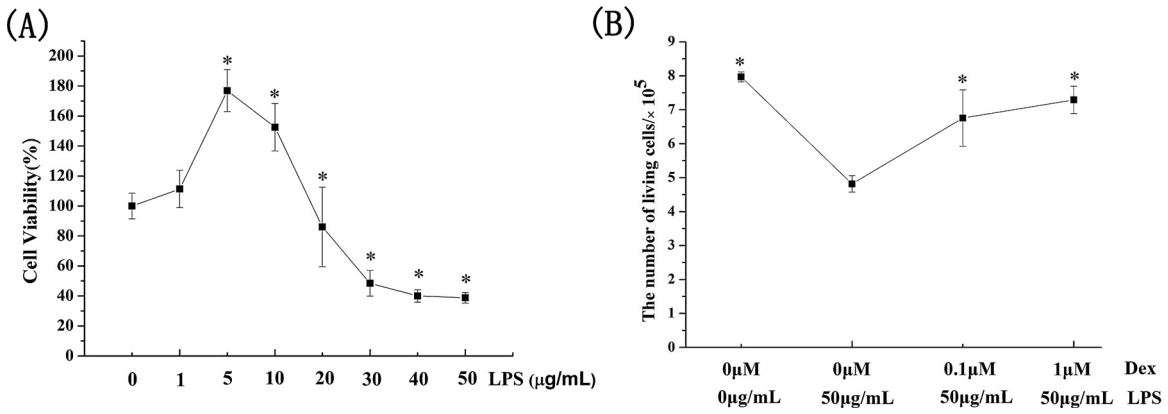

Fig. 5. (A) Effect of LPS on the viability of PIECs. (*Compare with control group (0 μg/mL LPS), $p < 0.05$). (b) Effect of different concentration of dexamethasone on the cytotoxicity induced in PIECs by LPS (50 μg/mL). (*Compare with a negative control group (50 μg/mL LPS, 0 μM Dex), $p < 0.05$).

3.4. *The protective effect of Dex on PIECs damaged by LPS*

Vascular endothelial cell layer as a intima maintained cardiovascular homeostasis and preserved good health. Endothelial dysfunction represents one of the earliest events in vascular, the protection of the endothelium is regarded as the most effective strategy to prevent the cardiovascular diseases [27].

In this study, the effect of LPS on PIECs viability was measured by MTT assay. PIECs (1×10^4) were cultured in normal medium 24 h, then treated with different concentration of LPS for 24 h, and the result showed that LPS induced the viability of PIECs reduction in a concentration-dependent manner as shown in Fig. 5A. It is interesting that the lower concentration of LPS (0–10 μg/mL) can promote the PIECs viability, however, higher concentration of LPS can inhibit cell growth. If the PIECs viability without LPS treatment was set as 100% as a control, the PIECs viability with 5 μg/mL LPS was higher than the control as 180%. But it was reduced to 40% when treated by 50 μg/mL LPS. The possible reason was that LPS can upregulate the expression of iNOS and the production of NO in endothelial cells, NO can inhibit LPS-induced endothelial cells apoptosis, however, high concentrations of NO can inhibit protein synthesis [7].

The protective effect of Dex on PIECs against the damage induced by LPS was detected by viable cell counts. PIECs (1×10^5 cells) were cultured in DMEM, containing 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin, and incubated at 37 °C for 24 h. Then medium was replaced with DMEM, containing 50 μg/mL LPS and different concentration of Dex and continuous culture for another 12 h, finally, the viable cells were counted as in Fig. 5B. The result showed that the cell number kept the highest in the control group (no Dex and LPS) (about 8×10^5 cells), LPS treated groups showed different cell viability based on the Dex addition amount. Without Dex, LPS treated group shown lowest cell viability (about 4.8×10^5 cells), but with the concentration of Dex increased the cell viability increased (6.7×10^5 cells (0.1 μM Dex) and 7.3×10^5 cells (1 μM Dex)). These results demonstrated that Dex can protect the PIECs from damage induced by LPS.

3.5. *The protective effect of Dex @SF/PEO nanofibers on PIECs damaged by LPS*

To verify the protective effect of Dex loaded nanofibers on PIECs damaged by LPS, the same number of PIECs (1×10^4) was seeded on four different groups of materials and the viability of cells were evaluated by MTT assay as shown in Fig. 6a. Cells seeded on (+) positive control grew the best among the four groups, because those were not treated with high concentration (50 μg/mL) LPS. But the

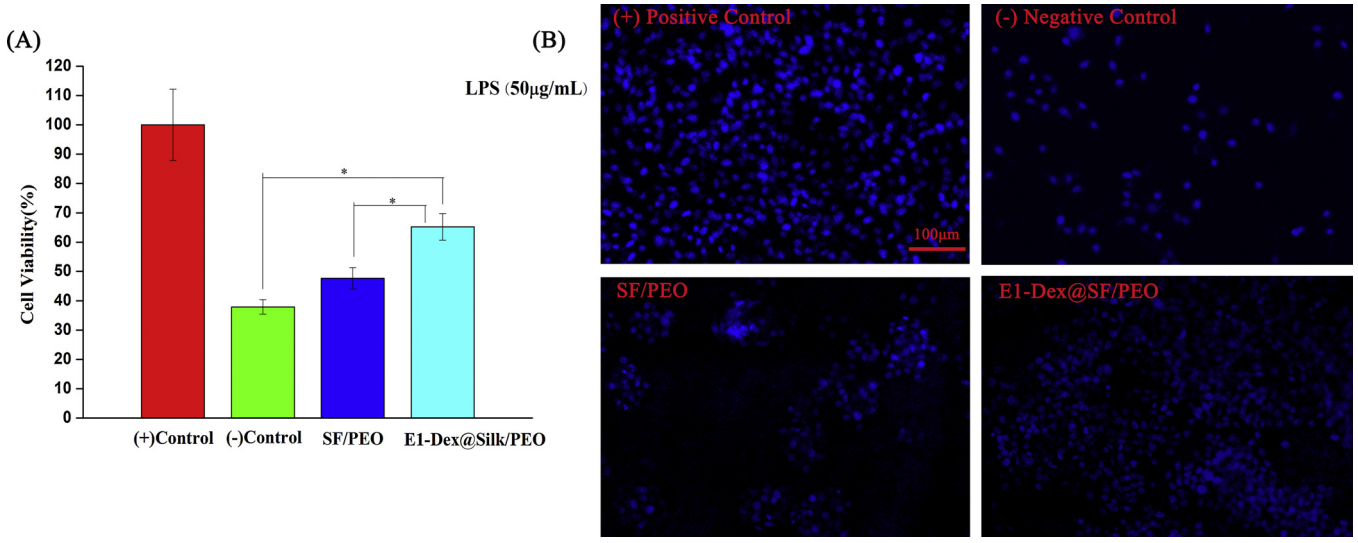

Fig. 6. (A) Evaluation of the effects of SF/PEO and E1-Dex@SF/PEO nanofibers on the viability of PIECs treated with LPS (50 μg/mL), viability was assessed by quantification using an MTT assay with data normalized to the untreated (+) positive control (assumed 100%). (B) On the right are confocal laser scanning microscopy images of PIECs seeded on different mats, blue represents the DAPI-stained cell nuclei. Significant differences between groups is indicated (*$p < 0.05$).

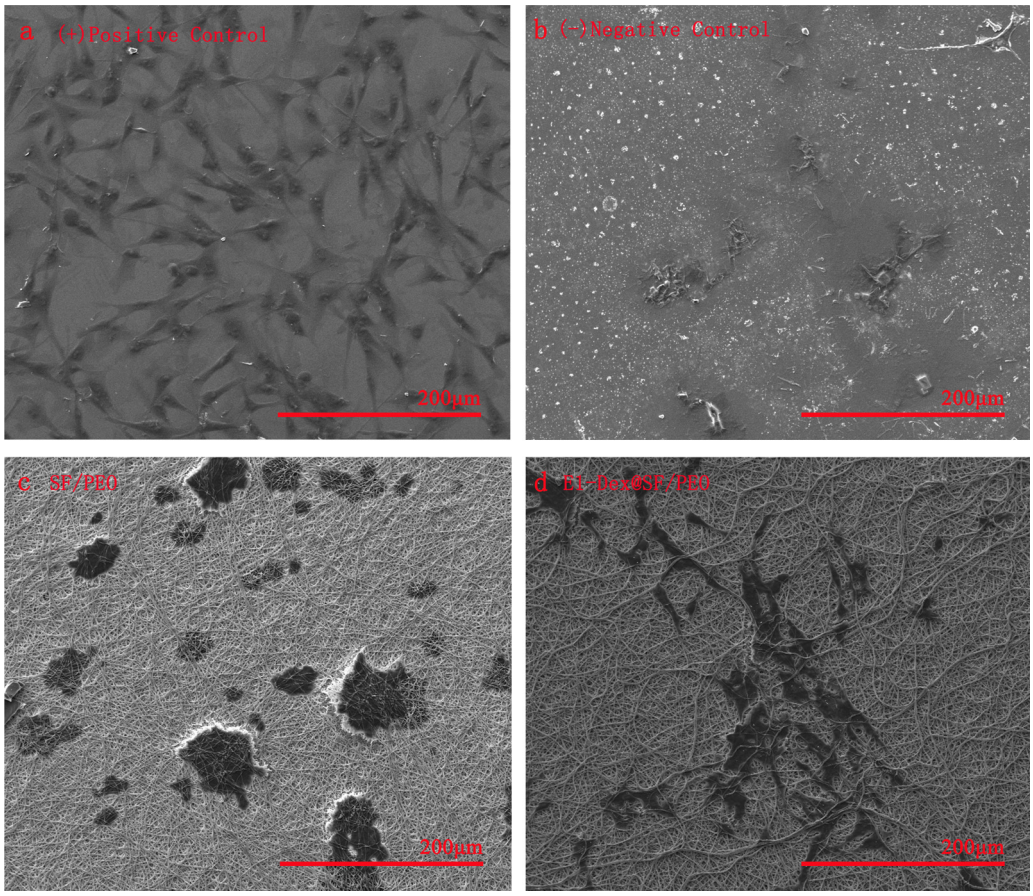

Fig. 7. SEM images of PIECs seeded on different mats, (a) (+) positive control mats, (b) (−) negative control mats, (c) SF/PEO nanofiber mats, (d) E1-Dex@SF/PEO nanofiber mats.

viability of cells, which seeded on (−) negative control group were only approximately 38% relative to the positive control (assumed as 100%), which demonstrated that those cells were badly damaged by LPS. Difference between negative group and SF/PEO group were not significant, indicating that the SF/PEO had a little protective effect on cells damaged. However, those cells seeded on E1-Dex@SF/PEO group grew much better than the negative control and SF/PEO nanofibers group, cells viability were approximately 65% which was 27% higher than negative control group. This clearly suggests that the Dex released from E1-Dex@SF/PEO nanofibers has an excellent protective effect on PIECs damaged by LPS.

To further confirm the protective effect of drug loaded nanofibers on PIECs which damaged by LPS, the morphological changes of cells seeded on different groups for 24 h were observed by CLSM and SEM. As shown in Fig. 6B) negative control showed the lowest viable cell number among the four groups. However, much more viable cells were found on E1-Dex@SF/PEO nanofiber mats.

The SEM result was shown in Fig. 7. The cells on the positive control were much more than three other groups, and the cells presented an extended morphology. However, cells on negative control were seriously damaged by LPS, not only the number of cells was less, the cell morphology was also changed, cellular debris were seen clearly. Cells seeded on neat SF/PEO nanofibers mats group grew better than negative control, but the cells acquired a round-shaped morphology and the cell number was reduced compared to the positive control group. However, the most important to note was that those cells seeded on E1-Dex@SF/PEO nanofibers presented an elongated and fibroblast-like morphology with similar morphology as in positive control. That further confirmed that the release of drug had a bioactivity and protected the PIECs from damage induced by LPS.

4. Conclusions

By emulsion electrospinning, Core–shell structured SF/PEO nanofibers can be obtained and Dex was successfully incorporated in the core part. In the process of electrospinning, organic solvent was discharged in consideration of its toxicity, so this nanofibers prepared method can be called “Green Electrospinning”. Dex loaded into the core of Dex@SF/PEO nanofibers prepared by emulsion electrospinning was released in a sustained and prolonged manner, *in vitro* experiment showed the released Dex can protect the PIECs from damage induced by LPS. SF/PEO nanofiber mat is a potential drug carrier for blood tissue engineering and skin tissue engineering. Moreover, some other hydrophobic drugs may also be incorporated into the SF/PEO nanofibers and loaded into the core.

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References

[1] S. Agarwal, A. Greiner, Polym. Adv. Technol. 22 (2011) 372–378.
[2] J. Sun, K. Bubel, F. Chen, T. Kissel, S. Agarwal, A. Greiner, Macromol. Rapid Commun. 31 (2010) 2077–2083.
[3] M. Wang, J.H. Yu, D.L. Kaplan, G.C. Rutledge, Macromolecules 39 (2006) 1102–1107.
[4] H.-J. Jin, J. Park, R. Valluzzi, P. Cebe, D.L. Kaplan, Biomacromolecules 5 (2004) 711–717.
[5] C. Li, C. Vepari, H.-J. Jin, H.J. Kim, D.L. Kaplan, Biomaterials 27 (2006) 3115–3124.
[6] L. Fan, H. Wang, K. Zhang, Z. Cai, C. He, X. Sheng, X. Mo, RSC Adv. 2 (2012) 4110–4119.
[7] D.D. Bannerman, S.E. Goldblum, Am. J. Physiol.-Lung Cell. Mol. Physiol. 284 (2003) L899–L914.
[8] R. Winn, J. Harlan, J. Thromb. Haemost. 3 (2005) 1815–1824.
[9] R. Palmer, L. Bridge, N. Foxwell, S. Moncada, Br. J. Pharmacol. 105 (1992) 11–12.
[10] R. Korhonen, A. Lahti, M. Hämäläinen, H. Kankaanranta, E. Moilanen, Mol. Pharmacol. 62 (2002) 698–704.
[11] U.K. Meßmer, G. Winkel, V.A. Briner, J. Pfeilschifter, Br. J. Pharmacol. 129 (2000) 1673–1683.
[12] N.M. Vacanti, H. Cheng, P.S. Hill, J.O.D. Guerreiro, T.T. Dang, M. Ma, S.E. Watson, N.S. Hwang, R. Langer, D.G. Anderson, Biomacromolecules 13 (2012) 3031–3038.
[13] L. Fan, H. Wang, K. Zhang, C. He, Z. Cai, X. Mo, J. Biomater. Sci. Polym. Ed. 23 (2012) 497–508.
[14] L. Xiaoqiang, S. Yan, C. Rui, H. Chuanglong, W. Hongsheng, M. Xiumei, J. Appl. Polym. Sci. 111 (2009) 1564–1570.
[15] A. Rawat, D.J. Burgess, Int. J. Pharm. 415 (2011) 164–168.
[16] H.-J. Jin, J. Chen, V. Karageorgiou, G.H. Altman, D.L. Kaplan, Biomaterials 25 (2004) 1039–1047.
[17] J. Wu, C. Huang, W. Liu, A. Yin, W. Chen, C. He, H. Wang, S. Liu, C. Fan, G.L. Bowlin, J. Biomed. Nanotechnol. 10 (2014) 603–614.
[18] P. Gupta, C. Elkins, T.E. Long, G.L. Wilkes, Polymer 46 (2005) 4799–4810.
[19] J. Zeng, X. Xu, X. Chen, Q. Liang, X. Bian, L. Yang, X. Jing, J. Control. Release 92 (2003) 227–231.
[20] T.J. Sill, H.A. von Recum, Biomaterials 29 (2008) 1989–2006.
[21] Y. Su, Q. Su, W. Liu, M. Lim, J.R. Venugopal, X. Mo, S. Ramakrishna, S.S. Al-Deyab, M. El-Newehy, Acta Biomater. 8 (2012) 763–771.
[22] X. Xu, L. Yang, X. Xu, X. Wang, X. Chen, Q. Liang, J. Zeng, X. Jing, J. Control. Release 108 (2005) 33–42.
[23] H. Qi, P. Hu, J. Xu, A. Wang, Biomacromolecules 7 (2006) 2327–2330.
[24] Y. Yang, X. Li, M. Qi, S. Zhou, J. Weng, Eur. J. Pharm. Biopharm. 69 (2008) 106–116.
[25] X. Li, Y. Su, S. Liu, L. Tan, X. Mo, S. Ramakrishna, Colloids Surf. B: Biointerfaces 75 (2010) 418–424.
[26] A. Yarin, Polym. Adv. Technol. 22 (2011) 310–317.
[27] Y. Shan, R. Zhao, W. Geng, N. Lin, X. Wang, X. Du, S. Wang, Cardiovasc. Toxicol. 10 (2010) 139–145.