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Distribution of Sorbitan Monooleate in poly(L-lactide-co-ε-caprolactone) nanofibers from emulsion electrospinning

Xiaoqiang Li[a,b,c], Yan Su[a,b,c], Xu Zhou[c], Xiumei Mo[a,b,c,*]

[a] State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai 201620, China
[b] College of Materials Science and Engineering, Donghua University, Shanghai 201620, China
[c] Biomaterials and Tissue Engineering Laboratory, College of Chemistry, Chemical Engineering and Biotchnology, Donghua University, Shanghai 201620, China

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ABSTRACT

The aim of this study was to investigate the distribution of Sorbitan Monooleate (Span80) in poly(L-lactide-co-ε-caprolactone) (PLLACL) nanofibers from emulsion electrospinning. The hypothesis was that PLLACL/Span80 nanofibrous mats would have some Span80 on the surface of the composite nanofibers. To test the hypothesis, the electrospinning of emulsions made of PLLACL, chloroform, Span80, and distilled water to prepare PLLACL/Span80 nanofibers was systematically investigated. The morphology of PLLACL/Span80 nanofibers was investigated by atomic force microscopy. The surface hydrophilicity of the nanofibrous mats were examined by water contact angle test. The distribution of Span80 on the surface of nanofibrous mats was also confirmed by the performance of pig iliac endothelium cells on the nanofibrous mats.

1. Introduction

Electrospinning is a technique that utilizes electric force alone to drive the spinning process and to produce polymer fibers from solutions or melts [1–3]. Unlike conventional spinning techniques (e.g., solution- and melt-spinning), which are capable of producing fibers with diameters in the micrometer range, electrospinning is capable of producing fibers with diameters in the nanometer range. Electrospun polymer nanofibers possess many extraordinary properties including small diameters and the concomitant large specific surface areas, a high degree of structural perfection and the resultant superior mechanical properties. Additionally, the nonwoven fabrics (mats) made of electrospun polymer nanofibers offer a unique capability to control the pore sizes among nanofibers. Unlike nanorods, nanotubes, and nanowires that are produced mostly by synthetic, bottom-up methods, electrospun nanofibers are produced through a top-down nano-manufacturing process, which results in continuous and low-cost nanofibers that are also relatively easy to be aligned, assembled and processed into applications.

Recently, electrospinning of emulsions (instead of solutions) to prepare core–shell type of polymer nanofibers have attracted growing interests [4–7]. The emulsions (particularly water-in-oil type of emulsions) usually contain an oil phase that is a polymer (e.g., a biocompatible and/or biodegradable polymer) dissolved in an organic solvent, and a water phase (emulsion particles with sizes of microns and/or sub-microns) that is a bioactive substance (e.g., a drug) dissolved in water. The emulsion particles need to be stabilized by an emulsifier or a surfactant. Electrospinning of emulsions can result in the formation of the core–shell type of nanofibers made of drugs encapsulated in biocompatible/biodegradable polymers. Such core–shell type of nanofiber mats possesses the combined characteristics of a scaffold and a controllable drug releasing agent. Furthermore, the properties of the core–shell nanofiber mats can be tailored by judiciously selecting polymers, drugs, emulsifiers or surfactants, and electrospinning processing conditions. Nonetheless, the added emulsions/surfactants located only at core part of electrospun nanofibers remains to be a critical concern. For example, Xu et al. revealed that emulsifier/surfactants help to form the "core–shell" structure nanofibers [8,9]. Additionally, the affect of emulsion on the morphology of nanofibers need to be further investigated.

The aims of this study were to investigate the distribution of Sorbitan Monooleate (Span80) in electrospun poly(L-lactide-co-ε-caprolactone) (PLLACL) nanofibers which were expected to prepare core–shell type of nanofibers. PLLACL was selected for this study because it has been widely investigated for biomedical applications. For example, our previous research revealed that both smooth muscle cells and endothelial cells could well anchorage and proliferate on the electrospun PLLACL nanofibrous mats [10], and the presence of Span80 have no negative affect for the growing

* Corresponding author at: Institute of Biology Science and Biotechnology, Donghua University, Shanghai 201620, PR China.
E-mail address: xmm@dhu.edu.cn (X. Mo).

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of endothelial cells. In this study, the PLLACL nanofibers contained Span80 were successfully prepared by electrospinning emulsions made of PLLACL, chloroform, Span80, and distilled water. For comparison purpose, the plain PLLACL nanofiber mats (without Span80) were also prepared as the control samples. The morphologies of the prepared nanofiber mats were examined by atomic force microscopy (AFM); the surface hydrophilicity was measured by the water contact angle method; and the distribution of Span80 was investigated by the performance of pig iliac endothelium cells (PIECs) on the nanofiber mats.

2. Materials and methods

2.1. Materials

PLLACL with molar ratio of 50% being L-lactide was purchased from the Sigma–Aldrich Co. (Milwaukee, Wisconsin). Span80 was purchased from the Zibo Haijie Chemical Industry Co., Ltd. (Zibo, China). Chloroform was purchased from the Shanghai Fine-Chemicals Co., Ltd. (Shanghai, China). All culture media and reagents were purchased from the Gibco Life Technologies Co. (Carlsbad, California). All of the materials and reagents were used without further purification.

2.2. Preparation of emulsions

Span80 (0.04 ml) was first dissolved in 10 ml chloroform; subsequently, 2 wt.% distilled water was added dropwise; this was followed by stirring the mixtures at 240 rpm for 20 min to obtain uniform emulsions. PLLACL was then added into the emulsions, dissolved in the chloroform phase, and the concentration of PLLACL in the emulsions was 6 wt.%. As the control samples, PLLACL alone (no Span80) was also dissolved in chloroform (no water) to prepare solutions with the same concentrations of 6 wt.%.

2.3. Electrospinning of nanofibrous mats

The experimental set-up used for conducting electrospinning included a high voltage power supply (BGG DC high-voltage generator), purchased from the BMEI Co., Ltd. (Beijing, China), and a digitally controlled and extremely accurate syringe pump (KDS 200), purchased from KD Scientific (Holliston, Massachusetts). During electrospinning, a positive high voltage of 20 kV was applied at the tip of a syringe needle with the inner diameter of 0.9 mm. The electrospun nanofibers were collected on a piece of aluminum foil covered on an electrically grounded metal plate, which was placed at a distance of 12 cm below the tip of the syringe needle. The mass flow rate was maintained at 1.0 ml/h. The electrospinning was conducted under the ambient conditions.

2.4. Morphologies of nanofibrous mats

Surface morphology of the nanofibers was examined using a Nanoscope IV atomic force microscope (Veeco Instruments Inc., USA), in the tapping mode and expressed as height and phase images.

2.5. Water contact angles of nanofibrous mats

Water contact angles of nanofibrous mats were measured using a contact angle analyzer made by the Data Physics Corp. (San Jose, California) to identify the effect of Span80 addition on the hydrophilicity of electrospun nanofiber mats. During the measurements, the samples of nanofiber mats were first cut into square pieces with the size of 1 cm^2, followed by placing them on a testing plate. Subsequently, 0.03 ml distilled water was carefully dropped onto the prepared specimens. The contact angles between water droplets and nanofiber mats were measured by taking photos at various time periods (0, 1, 2, 3, 4, 5, 6 and 8 s). Three measurements at different positions were carefully conducted for each specimen; and the reported data were the mean value with the error bar representing one standard deviation.

2.6. PIECs performance on the electrospun PLLACL/Span80 nanofibrous mats

PIECs (purchased from Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences, Shanghai, China) were cultured in the Dulbecco/Vogt modified Eagle's minimal essential medium (DMEM) with 10 wt.% fetal bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin in a humidified incubator with the CO_2 content being 5 vol% and the temperature being 37 °C. The medium was refreshed every 3 days. Medical-grade cover-slips (14 mm in diameter) were placed on the aluminum foil to collect the nanofiber mats during electrospinning. The slips (covered with ~50 μm thick nanofiber mats) were then fixed on 24-well plates by stainless steel rings, and were further sterilized with 75 vol% alcohol solution. After sterilization, the samples were washed with the phosphate-buffered saline solution (PBS) for 2 h.

PIECs were seeded onto nanofibrous mats at a density of 5.0×10^4 cells/cm^2 for cell morphology by scanning electron microscopy (S-2700, Hitachi, Japan). The cells cultured on the cover slips and the mats were washed with PBS and then fixed with 4% glutaraldehyde for 45 min at 4 °C. Thereafter, the samples were dehydrated in 50, 75 and 100% alcohol solutions, and dried under vacuum. The samples were sputter coated with gold and observed under SEM at a voltage of 15 kV.

3. Results and discussion

3.1. Morphology of electrospun nanofibers with and without Span80

In the electrospinning system, there are a number of parameters affecting fiber morphology and fiber diameter, such as polymer concentration/viscosity, applied voltage, needle diameter and the delivery rate of polymer solution [11]. Additionally, the solutes used to make the polymer solution have a significant effect on the result fibers morphology.

In this study, it was found that the surface morphologies of PLLACL nanofibers changed with the addition of Span80. As shown in Fig. 1, the morphology of PLLACL nanofibers with and without Span80 was examined by AFM. From the analysis of AFM, it is obviously observed that the surface of PLLACL/Span80 nanofibers was much smooth than plain PLLACL nanofibers. On the surface of plain PLLACL nanofiber, there were a lot of undulations. On the other hand, the PLLACL/Span80 nanofiber presents a smooth surface as contrast.

3.2. Water contact angles of nanofibers mats

The hydrophilicity of nanofiber mats could play an important role to determine their overall performances as scaffolds to grow cells, and was characterized in this study by the water contact angle method. The detailed procedures have been described in Section 2. As shown in Fig. 2, water contact angle variations after the water droplets were placed on the nanofibrous mats for 0, 1, 2, 3, 4, 5, 6 and 8 s. It was evident that PLLACL/Span80 nanofibrous mats were much more hydrophilic than the Plain PLLACL nanofibrous mats. It is obviously to detected after the water droplets were placed on the nanofibrous mats for 4 s, the water contact angle of the PLLACL/Span80 nanofibrous mats was close to 0°, while that of the

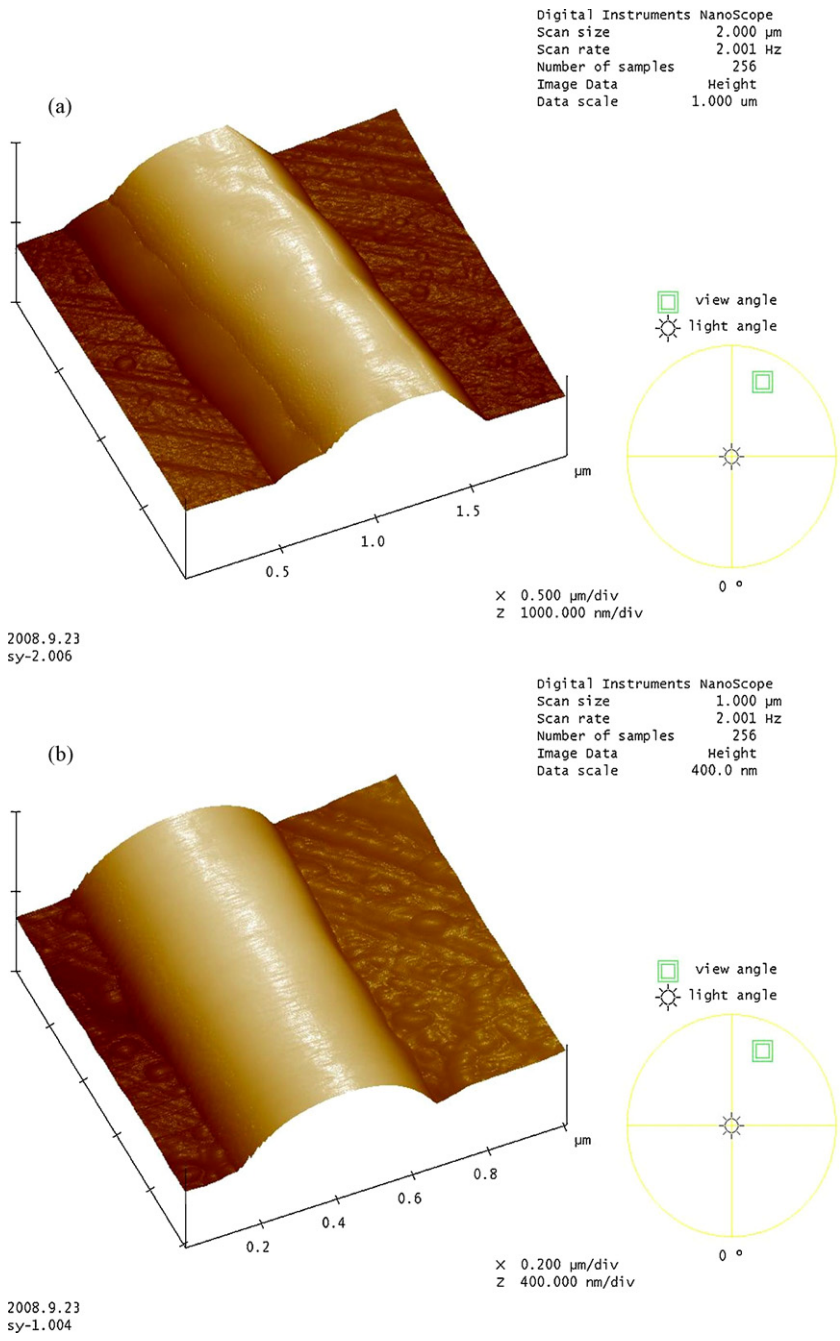

Fig. 1. Represented AFM images observed by height mode, (a) plain PLLACL nanofiber, and (b) PLLACL/Span80 nanofiber.

plain PLLACL nanofibrous mats was higher than 100°. It is the reasonable to get the conclusion that some Span80 molecules, which are much more hydrophilic than PLLACL, were locating on the surface of the PLLACL/Span80 nanofibrous mats while others existed as the nanorods inside the nanofibers.

3.3. *PIECs growth performance on nanofibrous mats*

For giving more evidences to test the hypothesis that some of Span80 located on the surface of electrospun nanofibers, the PIECs growth performances were investigated. It is easy to understand that animal cells had tendency to adhere on the biomaterials surface. As shown in Fig. 3a, PIECs anchorage on the PLLACL nanofibrous mat. It was found that PIECs were tending to grow by the leading of fibers orientation. However, if there were some small molecules, especially the surfactant molecules on the surface of nanofibers, the animal cells were difficult to attach and adhere on the nanofibrous mat. It is noted that both the nanofibrous mats which had PIECs growth on them were treated by alcohol solutions with different concentrations for several times before the examination of SEM (the details were described previously). The cells which adhered and grow on the PLLACL/Span80 nanofibrous mat were washed away by the treating of alcohol solutions, because the surfactant on nanofibers surface weaken the interforce between cells and nanofibers. Therefore, in Fig. 3b, there was no cell present on the PLLACL/Span80 nanofibrous mat, but only the growth prints. The hatchings on the nanofibrous mat were generated by PIEC cells growth behaviors, because animal cells would build the extracellular matrix during the growth process.

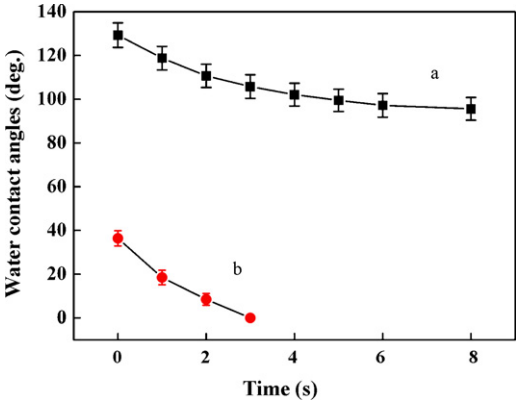

Fig. 2. The water contact angles of (a) PLLACL nanofibrous mats (symbol "■"), and (b) PLLACL/Span80 nanofibrous mats (symbol "●") after various seconds. Each datum is the mean value of three measurements with the error bar representing one standard deviation.

3.4. Span80 distribution in nanofibers

In order to elucidate the distribution of Span80 in emulsion electrospun nanofibers, a scheme was indicated as shown in Fig. 4. In the scheme, a simple pattern with a red head as the hydrophobic group and a curve tail as the hydrophilic group was designed to represent the surfactant of Span80. The concentric circles were the representation of cross section of PLLACL nanofiber. The aim of using emulsion electrospinning was to fabricate "core–shell" type nanofibers which had the potential to encapsulate drugs or proteins

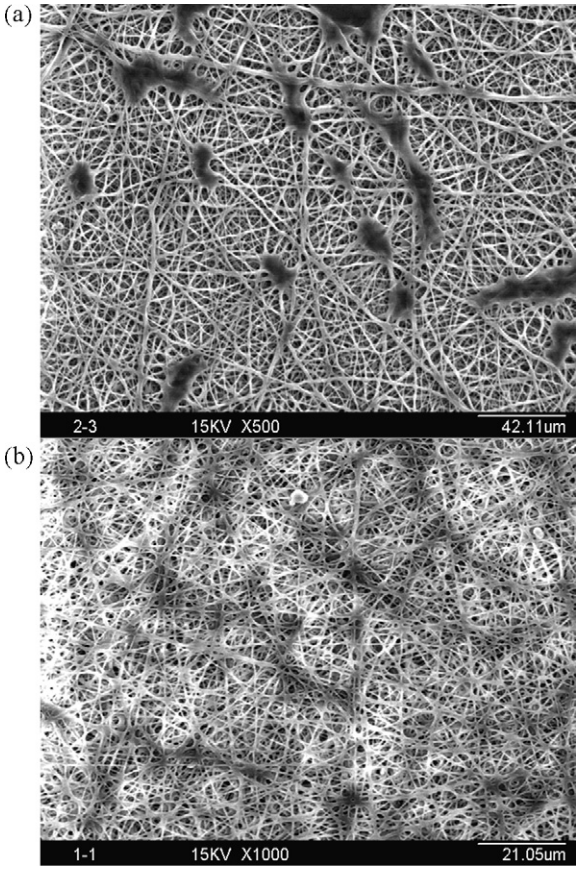

Fig. 3. SEM images represent PIECs growth performances on electrospun PLLACL nanofibrous mats with and without Span80. (a) PIECs growth on the surface of plain PLLACL nanofibrous mat after seeding for 24 h; (b) PIECs growth prints after seeding for 24 h, the hatchings on the nanofibrous mat were generated by PIEC cells growth behaviors.

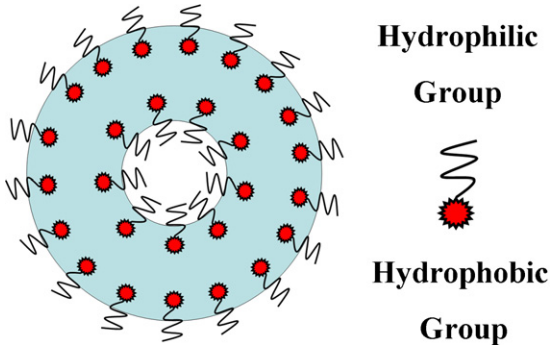

Fig. 4. Scheme of Span80 distribution in nanofiber cross section.

in the core part of nanofibers. The basic structure of "core–shell" type had been verified by other researchers and our previous study (the results had been written into a research article and will be published in Journal of Biomedical Materials Research: Part A). The surfactants (not on the surface of nanofibers) stabilize the nanofibers core part which encapsulate drugs or proteins. The other surfactants located on the surface of nanofibers as shown in Fig. 4.

4. Conclusions

The objectives of this study were to investigate the distribution of Span80 in emulsion electrospun PLLACL/Span80 nanofibers. The nanofibrous mats electrospun from emulsions could possess the unique combined characteristics of cell-growth scaffolds and controllable drug releasing agents. In this study, PLLACL/Span80 nanofibrous mats were successfully prepared by electrospinning of emulsions made of PLLACL, chloroform, Span80, and distilled water. Systematic investigations were carried out to study the morphology change of PLLACL nanofibers with and without Span80 in the nanofibers; and the optimal conditions were identified from AFM images. The incorporation of Span80 into PLLACL nanofibers resulted higher hydrophilicity can be explained by the reason that some Span80 molecules were on the surface of nanofibers. SEM examinations of PIECs growing condition also confirmed that the distribution of Span80 on the surface of nanofibers.

Acknowledgements

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