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Preparation of Doxorubicin-loaded Polylactic acid/graphene oxide nanofibrous membranes with different structures by electrospinning for cancer therapy

Short title: DOX-loaded PLA/GO nanofibrous membranes

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ABSTRACT

Background: Intravenous administration is still often used as adjuvant therapy after breast cancer resection, with toxic side effects. It is important to find a method of local administration to avoid the high cytotoxicity.

Methods: In the paper, a series of polylactic acid (PLA)-based nanofibers loaded with Doxorubicin (DOX) by blended and coaxial electrospinning were prepared to localize anticancer.

Results: Scanning electron microscope (SEM) shows that all nanofibers have smooth surfaces and uniform diameters, and DOX is uniformly dispersed in the fibers.

Conclusions: All electrospun fibers can effectively relieve the release of DOX and possess the ability to kill cells, and the ability of coaxial spinning is better than that of blended spinning.

Keywords: Doxorubicin; Electrospinning; Anticancer; Nanofibers

INTRODUCTION

For breast cancer, drug adjuvant therapy is often implemented to further remove residual cancer cells after resection, which can effectively avoid the metastasis and recurrence of residual cancer tissues, and improve the overall survival rate of patients.[1–3] Doxorubicin (DOX) and paclitaxel (PTX) are commonly used drugs,[4–7] almost all patients will experience side effects after taking the drug, such as bone marrow suppression, cardiotoxicity. Drugs are mainly administered by intravenous bolus injection. Before successfully reaching the lesion, the drug will inevitably cause damage to the normal organs and cells of the body during the delivery process. For patients having just undergone cancer tissue resection, the side effects caused by chemotherapy drugs undoubtedly aggravate the patient's suffering. So, the development of efficient drug delivery systems is quite important.

Fibers prepared by electrospinning have the characteristics of high specific surface area, high load capacity of drug and adjusted drug release rate,[8–10] which has promoted the development of tumor drug delivery. Electrospinning methods include blended and coaxial spinning.[11] The incorporation of drugs into the fibers by different methods can severely affect the drug release, so the optimal drug loading method for the desired application must be properly selected.

In this paper, DOX-loaded composite nanofibrous membranes with anticancer effects were prepared by blending and coaxial spinning. The drug-loaded nanofibers were characterized by Fourier transform infrared spectroscopy (FT-IR), SEM, and X-ray diffraction (XRD); the drug standard curve and release curves were determined by ultraviolet visible (UV-Vis) spectrophotometer. The cell models were carried out to evaluate the clinical application potential of drug-loaded composite nanofibrous

membranes.

EXPERIMENTAL SECTION

Materials

Graphene oxide (GO) was synthesized according to a published report.[12] Polylactic acid (PLA, Mn = 400,000 g/mol), Polyethylene glycol (PEG, Mn = 20,000 g/mol), N,-N dimethylformamide (DMF), and Dichloromethane (DCM) were bought from Sinopharm Chemical Reagents Co. Ltd., China. Doxorubicin hydrochloride (DOX·HCl), fetal bovine serum (FBS), and penicillin-streptomycin were supplied by Saen Chemical Technology Co., Ltd., Shanghai Sure Biotechnology Co., Ltd., and Hyclone Corporation, respectively. Human breast cancer cells MCF-7 were obtained from Zhongnan Hospital in Wuhan City, Hubei Province.

Preparation of spinning solution

Preparation of blended spinning solution

56 mg GO and 15.51 mg DOX·HCl were dispersed in 50 mL of a mixed solvent containing DMF and DCM with a volume ratio of 1:1 by ultrasound. 3.102 g PLA was dissolved in the solvent with continuous stirring at 25 ℃ under seal conditions. After PLA was dissolved, the solution was allowed to stand for one hour to remove bubbles in the liquid, which was marked as S15D. A solution without loading the drug was marked as S15. DOX-loaded PLA (PD) was prepared by PLA and DOX·HCl in the same way as S15D, and pure PLA dissolved was labeled as P.

Preparation of coaxial spinning solution

(1) Preparation of core solution.

56 mg GO was dispersed in 3.75 mL of the solution consisting of DMF and DCM with a volume ratio of 1:1 by ultrasound, which constitutes the core solution.

(2)Preparation of sheath layers.

15.51 mg DOX·HCl and 3.102 g PLA were dissolved in 50 mL of the mixed solution composed of DMF and DCM with a volume ratio of 1:1 to prepare the PLA solution, marked as C15R0D. To further control drug release, PEG was added to the sheath layer solution under the condition of a certain solute mass. The quality ratios of PEG and PLA are designed as PLA: PEG = 9:1 (w/w), PLA: PEG = 8:2 (w/w), and PLA: PEG = 7:3 (w/w), respectively. The resultant solutions were marked as C15R1D, C15R2D, and

C15R3D, respectively. C15R0, C15R1, C15R2, and C15R3 were prepared in the same way without adding DOX.

Fabrication of membranes

The membranes were prepared by electrospinning. The experiment was carried out under the following parameters: The positive and negative voltages are 8.5 kV and –2.5 kV, respectively. The bolus speed was 0.215 mm/min (in coaxial spinning, core: 0.015 mm/min, sheath: 0.2 mm/min), and the distance between positive and negative electrodes was 20 cm. After spinning for 2 h, each sample was taken out, put in a vacuum drying oven at 60 ℃, and dried for 24 h to remove residual solvent.

The standard curve and drug loading rate of drug-loaded membranes

The standard curve of DOX

The standard curve of DOX was attained by a series of tests on an ultraviolet spectrophotometer. The specific operation process includes the following steps: (1) The maximum absorption wavelength of DOX was measured and analyzed by an ultraviolet spectrophotometer, which confirmed that the optimal absorption wavelength was 480 nm. (2) DOX was dissolved in methanol to prepare DOX standard solutions and the absorbance at 480 nm was measured. (3) The curve was obtained when DOX concentration was as the abscissa and absorbance was as the ordinate. The curve was further linearly fitted to obtain the standard curve of DOX.

Drug release rate

1 g drug-loaded nanofiber membrane was immersed in a dialysis bag containing 5 mL of PBS (pH = 7.4) buffer. The dialysis bag was placed in a centrifuge tube containing 45 mL of PBS (pH = 7.4), and the tube was placed in a constant temperature shaking box at 37 ℃ with 100 r/min. According to the time point, 5 mL of the solution was taken and the content of the drug was determined by an ultraviolet spectrophotometer to further calculate the cumulative release rate. In order to make the next time point normal, 5 mL of fresh PBS was added to ensure the overall volume remained unchanged.

Cell Proliferation and Cell cytotoxicity

All the nanofiber membranes were soaked in the cell culture medium composed of 89% DMEM high glucose medium, 10% fetal bovine serum, and 1% penic-streptomycin at

11.3 mg/mL to prepare the extract (n = 6). Subsequently, the extracts irradiated with ultraviolet light for 2 h were poured into a 96-well plate with 2×10^3/mL of the cell density. The 10 μL of CCK-8 was added after cell culture for 24 h, 48 h, and 72 h, and then the cells were cultured in an incubator at 37 ℃ with 5% CO_2 for 2 h, and the absorbance at 450 nm was measured with a microplate reader.

Characterization of samples

A field emission scanning electron microscope (FESEM; Hitachi S-4800, Japan) was employed to observe the microstructures of nanofiber membranes. Fourier transform infrared spectra of drug-loaded membranes were studied using Fourier transform infrared spectroscopy (Thermo Nicolet, Thermo Scientific Corp., Massachusetts, USA). The crystallite structures of DOX and drug-loaded membranes were analyzed by XRD (Empyrean, PANalytical Corp., Almelo, Netherlands). A drug standard curve and release curves were determined by a UV spectrophotometer (UV-2550, China).

RESULTS AND DISCUSSION

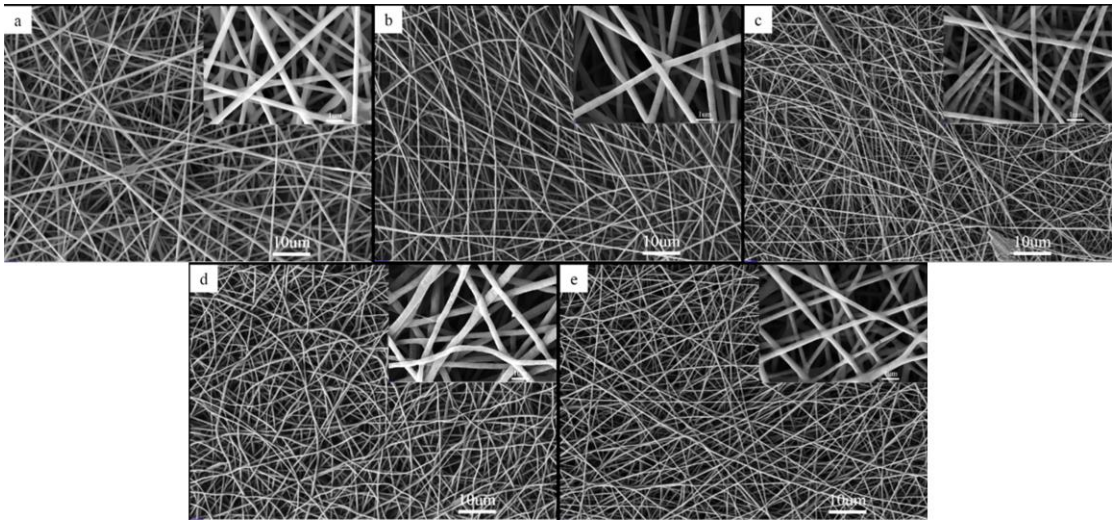

Figure 1. SEM images of the nanofibers: (a) PD; (b) S15D; (c) C15R0D; (d) C15R1D; (e) C15R2D.

Figure 1 is SEM images of nanofiber membranes loaded with DOX. Obviously, the surface of the nanofibers with uniform diameter is smooth, and particles are not observed on the surface of the fibers, which confirms that DOX is uniformly dispersed in the fibers. The diameters of the nanofibers become smaller with the increase of PEG. This is due to the viscosity of the spining solutions decreasing by increasing the content

of PEG and the solutions experiencing more bending instability to obtain the small diameter fibers.[13]

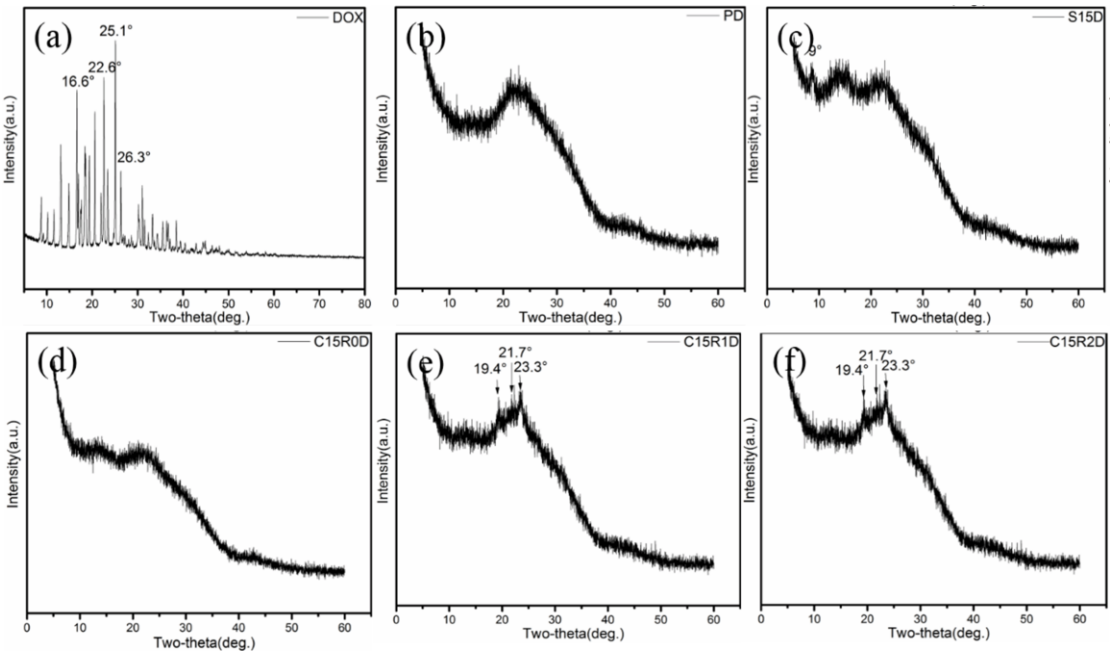

Figure 2. XRD patterns of DOX and the composite nanofibers: (a) DOX; (b) PD; (c) S15D; (d) C15R0D; (e) C15R1D; (f) C15R2D.

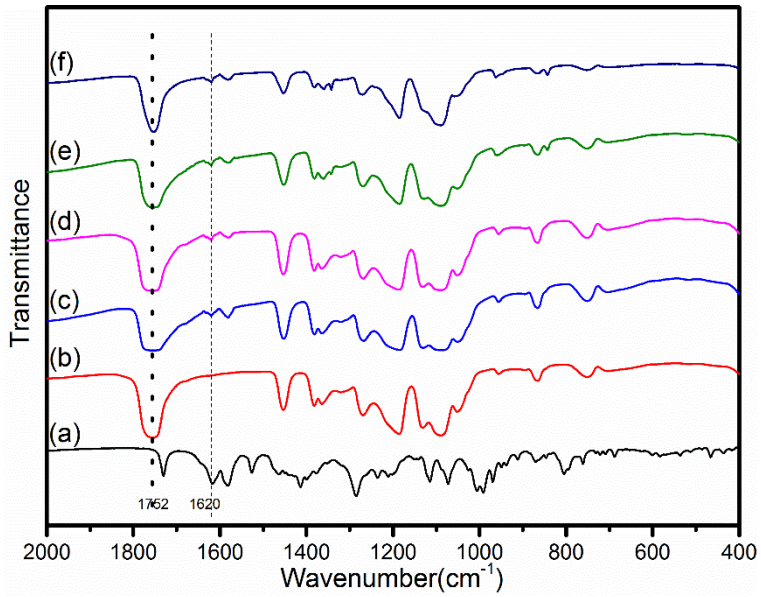

Figure 3. FT-IR spectra of DOX and the composite nanofibers: (a) DOX; (b) PD; (c) S15D; (d) C15R0D; (e) C15R1D; (f) C15R2D.

As shown in Figure 2, it is the XRD pattern of DOX and the DOX-loaded composite nanofibers. DOX has sharp diffraction peaks between 16.6° and 26.6° (Figure 2a),

suggesting that DOX exists in a crystalline state. The weaker crystalline peak of DOX can still be seen in the composite nanofibers with DOX. GO is present in S15D, C15R0D, and C15R1D and C15R2D. The crystalline peak of GO,[14] located at 9°, can only be seen in S15D. GO was coated on the core layer of C15R0D, C15R1D, and C15R2D, prepared by the coaxial co-spinning process. The structures weaken the intensity of its diffraction peaks, so no obvious diffraction peaks of GO are observed in Figures 3d-f. The peaks of C15R1D and C15R2D at 19.4°, 21.7°, and 23.3° correspond to the diffraction peaks of PEG,[15] which are not visible in C15R0D owing to the low content of PEG.

Figure 3 shows the FT-IR spectra of DOX and the nanofiber membranes with DOX. The peak of 1752 cm^{-1} is C = O of PLA.[16] Pure DOX has a characteristic functional group that exists at 1620 cm^{-1}, which is attributed to N-H bending vibration.[17] The absorption peak can also be observed in the composite nanofibers, which suggests that DOX has been successfully loaded into the nanofibers.

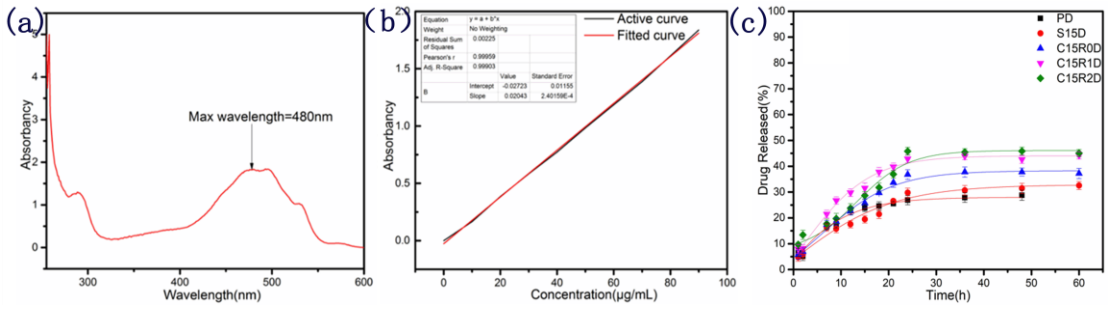

Figure 4. (a) UV-vis absorption spectrum of DOX; (b) The standard curve for DOX; (c) DOX release curves of nanofibers.

Figure 4a reveals the UV absorption spectrum of DOX, which has the largest absorption peak at 480 nm. Figure 4b is the standard curve of DOX obtained at the wavelength in the range of 0 μg/mL to 90 μg/mL. Absorbance and concentration have a good linear relationship (R^2 = 0.99903). Figure 4c shows the DOX release curves of nanofibers. C15R2D has the highest release rate and amount (45.09%) at 60 h. PEG of nanofibers can be dissolved in the buffer solution[18] to obtain a large number of microporous structures, forming a channel between the outside and the inside of the fiber, which is more conducive to drug release. The composite nanofiber membranes with different drug release rates indicate that the nanofibers with controlled drug release were successfully prepared.

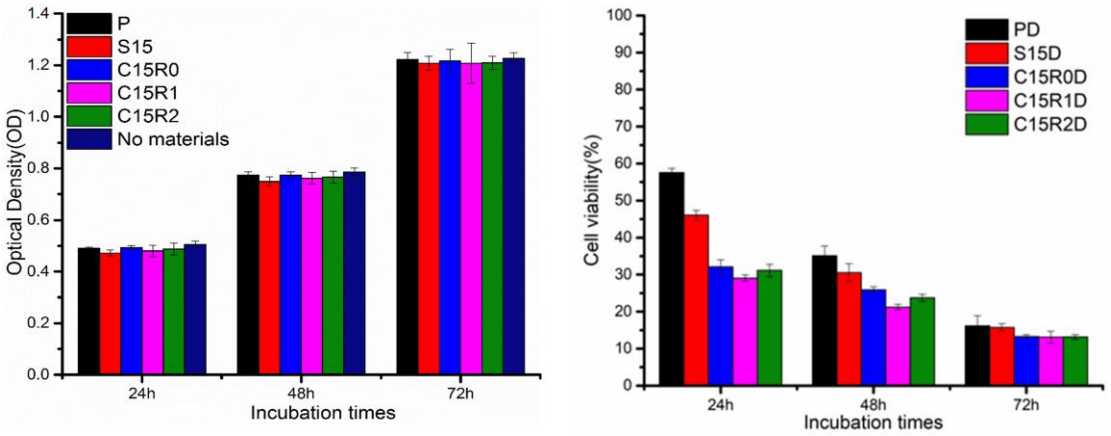

Figure 5. Cell proliferation of nanofibers: (a) without DOX; (b) with DOX.

Figure 5 shows the cell proliferation of the drug-loaded and non-drug-loaded composite nanofibers cells in the extract. For the fibers without DOX (Figure 5a), the OD values of all groups increase at each time point, indicating that the composite nanofibers have no obvious toxicity to human breast cancer cells MCF-7. For the fibers with DOX (Figure 5b), the cell survival rate of all groups was lower than 60% after 24 h incubation in the extract, indicating that DOX has an effective anticancer effect on breast cancer cells MCF-7. At the same time, the nanofibers with the core-shell structure show a better anticancer effect than the nanofibers made by blended electrospinning due to the faster drug release rate.

CONCLUSIONS

In summary, nanofibers with smooth surfaces and uniform diameters were successfully prepared by blending electrospinning and coaxial electrospinning. SEM, FT-IR spectra, and XRD patterns confirm that DOX is encapsulated in the nanofibers. The drug release curves confirm that the DOX-loading fibers can effectively alleviate the drug release, and the fiber prepared by coaxial electrospinning has better anticancer properties than the fiber prepared by blended electrospinning. This study provides a new idea for local drug delivery.

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Conflict of Interest

The authors declare no competing financial interest.

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