

Controlled Release of Doxorubicin from Electrospun MWCNTs/PLGA Hybrid Nanofibers*

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Abstract In this study, multiwalled carbon nanotubes (MWCNTs) were used to encapsulate a model anticancer drug, doxorubicin (Dox). Then, the drug-loaded MWCNTs (Dox/MWCNTs) with an optimized drug encapsulation percentage were mixed with poly(lactide-co-glycolide) (PLGA) polymer solution for subsequent electrospinning to form drug-loaded composite nanofibrous mats. The structure, morphology, and mechanical properties of the formed electrospun Dox/PLGA, MWCNTs/PLGA, and Dox/MWCNTs/PLGA composite nanofibrous mats were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy, and tensile testing. *In vitro* viability assay and SEM morphology observation of mouse fibroblast cells cultured onto the MWCNTs/PLGA fibrous scaffolds demonstrate that the developed MWCNTs/PLGA composite nanofibers are cytocompatible. The incorporation of Dox-loaded MWCNTs within the PLGA nanofibers is able to improve the mechanical durability and maintain the three-dimensional structure of the nanofibrous mats. More importantly, our results indicate that this double-container drug delivery system (both PLGA polymer and MWCNTs are drug carriers) is beneficial to avoid the burst release of the drug and able to release the antitumor drug Dox in a sustained manner for 42 days. The developed composite electrospun nanofibrous drug delivery system may be used as therapeutic scaffold materials for post-operative local chemotherapy.

Keywords: Electrospun nanofibers; Carbon nanotubes; Doxorubicin; Controlled release.

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INTRODUCTION

Development of drug delivery systems with controlled release properties has attracted a great deal of attention in the last few decades because the advanced drug delivery system enables improved therapeutic efficacy, low side effects, and convenient administration in comparison with conventional dosage forms. To build an ideal controlled release drug delivery system, various kinds of nano-sized vehicles have been developed, such as nanoparticles^[1, 2], nanocapsules^[3, 4], nanotubes^[5], dendrimers^[6, 7], and nanogels^[8]. Very recently, with the

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development of electrospinning techniques^[9], electrospun nanofibers have been explored as novel viable drug delivery vehicles and display prominent advantages in tissue engineering scaffold fields and post-operative local chemotherapy^[10–12].

The recurrence of tumor after resection is a common occurrence because some invisible metastases may happen during resection, which may cause an intractable clinical problem for the cure of cancer. Commonly, patients are recommended to take chemotherapy for a period of time in order to eliminate remaining cancer cells and to decrease the incidence of post-surgery of tumors. In a systemic chemotherapy, a large dosage or an increased number of administration dosages is required to achieve the desirable therapeutic effect, but this may cause severe side effects to normal tissues. Therefore, the electrospun nanofibers seem to be a promising carrier system to provide localized delivery of anticancer drugs to resection areas. Firstly, the drug-loaded electrospun nanofibers may maximize the drug concentration in tumors and minimize the toxicity to normal tissues. Moreover, compared with the systemic method, the drug-loaded electrospun nanofibers with sustained release property may increase in the duration of tumor exposure to anticancer drugs at cytotoxic concentrations at much lower total doses^[13–15]. Finally, electrospun nanofibers can be easily fabricated to have different shapes for clinical applications and possess 3D structure with high porosity mimic to the natural extracellular matrix (ECM), providing great convenience for the recovery of injured organs^[16, 17]. Therefore, development of electrospun nanofiber-based drug delivery system for cancer treatment has attracted much attention in recent years.

So far, a number of different anticancer drug-loaded electrospun nanofibers have been developed and displayed encouraging results^[18–22]. For example, paclitaxel-loaded polylactide-co-glycolide (PLGA) micro- and nanofibers were fabricated by blend electrospinning, and the composite fibers could release drug in a sustained manner and show comparable IC50 value to the commercial paclitaxel formulation-Taxol^[23]. Cisplatin-loaded poly(L-lactic acid) fibers displayed much higher antitumor activity than the free cisplatin^[24], and hydroxycamptothecin (HCPT)-loaded poly(DL-lactic acid)-poly(ethylene glycol) fibrous mats showed over 7 times higher inhibitory activity against cancer cells than the free drug^[18]. Moreover, implanted paclitaxel-loaded PLGA nanofibers discs displayed optimal paclitaxel pharmacokinetics in the brain/tumor with significant tumor inhibition in a glioblastoma xenograft model in mice^[25], and doxorubicin (Dox)-loaded polylactide electrospun nanofibers were demonstrated to be an effective local chemotherapy system against secondary hepatic carcinoma^[26]. However, the burst release effect at the initial period is often unavoidable and the mechanical property of drug-loaded nanofibers is weak, quite limiting their further biomedical applications.

In a previous work, we combined biocompatible inorganic nanomaterials, such as halloysite nanotubes, laponite, and hydroxyapatite with biodegradable polymer PLGA to form electrospun composite nanofiber-based drug delivery system^[19, 27–29]. Inorganic nanomaterials were used to encapsulate model drugs, and then the drug-loaded nanomaterials were mixed with PLGA solution for subsequent electrospinning to form composite drug-loaded nanofibers. Since both the nanomaterials and PLGA fiber matrix can act as drug delivery carriers, the developed double-container drug delivery system has been proven to be able to reduce the drug release rate and significantly suppress the initial burst release. Moreover, the mechanical property of composite nanofibers could be improved significantly after the doping of inorganic nanomaterials in nanofibers.

In this study, doxorubicin (Dox), one of the most widely employed chemotherapeutic drugs in cancer treatment, was chosen as the model drug and loaded in carboxyl-functionalized multiwalled carbon nanotubes (MWCNTs), which acted as the inorganic nanomaterials of the delivery system. And then the drug-loaded composite nanofibers (Dox/MWCNTs/PLGA) were fabricated by electrospinning the mixture solution of drug-loaded MWCNTs and PLGA. As a control, the conventional type of Dox-loaded PLGA nanofibers (Dox/PLGA) was also prepared by electrospinning mixture solution of Dox and PLGA. The formed nanofibers were characterized using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and mechanical testing. The biocompatibility of the electrospun nanofibrous scaffold was investigated through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay and SEM morphology

observation of the mouse fibroblasts cultured onto the MWCNTs/PLGA nanofibrous scaffolds. The drug release kinetics of the composite nanofibers was also investigated. To our knowledge, this is the first report related to the release property study of the electrospun MWCNTs-doped composite drug delivery system.

EXPERIMENTAL

Materials

PLGA ($M_w = 8.1 \times 10^4$ g/mol) with a lactic acid/glycolic acid ratio of 50:50 were purchased from Jinan Daigang Bio-technology Co., Ltd. (China). MWCNTs (diameter = 30–70 nm, length = 100–400 nm) with carboxyl residues (MWCNT-COOH) were obtained from University of Michigan (Ann Arbor, MI). Tetrahydrofuran (THF) and *N,N*-dimethyl formamide (DMF) were from Sinopharm Chemical Reagent Co., Ltd. (China). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was purchased from Daikin Industries (Japan). Mouse fibroblasts were from Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). Unless otherwise specifically explained, all cell culture medium and reagents were purchased from Hangzhou Jinuo Biomedical Technology Co., Ltd. (Hangzhou, China). Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18 M Ω ·cm.

Preparation and Optimization of the Loading of Dox onto MWCNTs

MWCNTs were dispersed in an aqueous solution at a concentration of 2 mg/mL and the solution pH was adjusted to be 9.0 using NaOH (0.1 mol/L). Dox was also dissolved in an aqueous solution with a concentration of 2 mg/mL (pH = 9.0). The Dox solution was mixed with the CNT solution ($V/V = 1:1$). pH of the mixture was adjusted to 7.0, 8.0, 9.0 or 9.5 using NaOH (0.1 mol/L) or HCl (0.1 mol/L) in order to evaluate the influence of pH on the loading efficiency of Dox onto MWCNTs. The mixture was stirred for 24 h at room temperature. The products were collected by centrifugation and washed several times with PBS until the supernatant became color free. The drug-loaded MWCNTs were dried in an oven for 12–24 h at 50 °C to reach a constant weight. The dried powders were ground and sieved.

The supernatants in all the rinsing steps were collected and the amount of unbound Dox in the solution was determined by measuring the absorbance at 481 nm *via* a Lambda 25 UV-Vis spectrophotometer (Perkin Elmer, Boston, MA) using a calibration curve prepared under the same condition. The loading efficiency was calculated according to the following equation:

$$\text{Loading efficiency (\%)} = \frac{\text{weight of (initial drug - unbound drug)}}{\text{weight of initial drug}} \times 100\% \quad (1)$$

The pH value used to load Dox onto the MWCNTs was optimized according to the loading efficiency of Dox onto the MWCNTs.

Preparation of Electrospun MWCNTs/PLGA Composite Nanofibers

PLGA (0.25 g/mL) was dissolved in a mixed solvent of THF/DMF ($V/V = 3:1$), MWCNTs (3 wt% or 5 wt% relative to PLGA) were mixed with PLGA solution for subsequent electrospinning. Electrospinning was carried out under ambient conditions. The electrospinning parameters were similar to our previous work (applied voltage = 20 kV, tip to collector distance = 15 cm, feeding speed = 0.8 mL/h). The formed fibrous mats were vacuum dried for at least 2 days at ambient temperature to remove the residual organic solvent and moisture.

Preparation of Electrospun Dox/MWCNTs/PLGA Composite Nanofibers

Firstly, drug-loaded Dox/MWCNTs with an optimized loading efficiency of 83.7% were prepared. Then PLGA (0.25 g/mL) was dissolved in a mixed solvent of THF/DMF ($V/V = 3:1$), and Dox/MWCNTs (with 1 wt%, 2 wt% or 3 wt% of Dox relative to PLGA) were mixed with PLGA solution for subsequent electrospinning according to the above conditions. DC-1/PLGA, DC-2/PLGA, or DC-3/PLGA was used to denote samples Dox/MWCNTs/PLGA with 1 wt%, 2 wt% or 3 wt% of Dox relative to PLGA, respectively. Moreover, electrospun Dox/PLGA nanofibers were also prepared. Dox was first dispersed in HFIP, and mixed with a small quantity of triethylamine to make Dox well dissolved. The Dox (1 wt% relative to PLGA) solution was then

added into PLGA solution with continuous stirring for 1 h until a clear and homogeneous solution was obtained.

Characterization Techniques

Morphologies of PLGA, MWCNTs/PLGA, Dox/MWCNTs/PLGA, and Dox/PLGA nanofibers were observed using SEM (JEOL JSM-5600LV, Japan). The accelerating voltage was set at 15 kV. Before SEM observations, the samples were sputter-coated with gold films with a thickness of 10 nm. The diameters of the electrospun fibers were analyzed using ImageJ 1.40G software (<http://rsb.info.nih.gov/ij/download.html>, National Institutes of Health, USA). At least 200 nanofibers at different images were analyzed for each sample. The mechanical properties of the electrospun fibrous mats were examined using a materials testing machine (H5K-S, Hounsfield, UK) with an elongation speed of 10 mm/min at 20 °C and a relative humidity of 63%. Before measurements, specimens were cut into rectangular pieces with the size of 10 mm × 50 mm. The thickness of each specimen was measured with a micrometer. In the test, the two ends of sample were gripped in the top and bottom chucks, respectively, and the gauge of two chucks connected to testing machine was fixed at 30 mm. The tensile testing was performed with a load cell of 10 N. Stress and strain were calculated through the following equations:

$$\sigma = \frac{P}{w \times d} \quad (2)$$

$$\varepsilon = \frac{l}{l_0} \times 100\% \quad (3)$$

where σ , ε , P , w , d , l and l_0 stand for stress (MPa), strain, load (N), mat width (mm), mat length (mm), extension length, and gauge length, respectively. Breaking strength, failure strain and Young's modulus were obtained from the strain-stress curves.

In vitro Drug Release

The release property of Dox/MWCNTs and Dox/MWCNTs/PLGA was investigated in both physiological and acidic environment. Acetate buffer (pH = 5.4) and PBS (pH = 7.4) were chosen as the release medium. To test the drug release from the MWCNTs, appropriate amount of Dox/MWCNTs powder was dispersed into 2 mL of acetate buffer (pH = 5.4) and PBS (pH = 7.4), respectively. Then the dispersed solution was loaded into a dialysis tube and dialyzed against 15 mL acetate buffer and PBS, respectively. For electrospun nanofibrous samples, only acetate buffer (pH = 5.4) was used. The composite nanofibrous mats, *i.e.* Dox/PLGA, DC-1/PLGA, DC-2/PLGA or DC-3/PLGA, were cut into three pieces in parallel and their weights were accurately measured to ensure the absolute amount of Dox was similar to that in Dox/MWCNTs powder. The samples were then placed in different vials containing 15 mL of acetate buffer. All the samples for release experiments were incubated in a vapor-bathing constant temperature vibrator at 37 °C. At a selected time interval, 3 mL of solution was taken out from the vials and equal volume of corresponding fresh buffer was replenished. The release kinetics of Dox was evaluated using a UV-Vis spectrophotometer. The characteristic absorption peak of 481 nm for Dox was monitored.

Cytocompatibility Assay of Composite Nanofibrous Mats

The cytocompatibility of electrospun PLGA and MWCNTs/PLGA nanofibers were estimated through MTT assay and SEM observation of L929 cells. The cells were cultured in 25 cm² tissue culture flasks with 5 mL of DMEM containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin. The medium was replaced every 3 days and the cell culture was maintained in an incubator with 5% CO₂ at 37 °C. Cells were seeded onto cover slips, tissue culture plates (TCPs), and electrospun PLGA, MWCNTs/PLGA (with 3 wt% and 5 wt% of MWCNTs relative to PLGA) nanofibrous scaffolds, respectively. All of these substrates were fixed in a 24-well tissue culture plate in triplicate. Cell seeding density was set at 1.0×10^4 and 2.0×10^4 per well for cell proliferation assay and SEM observation, respectively, according to the literature^[30]. The viability of cells cultured onto these substrates was quantitatively evaluated through MTT assay after cell seeding for 3 days. The

MTT assay was performed using a Microplate Reader (MK3, Thermo, Waltham, MA) according to the manufacture's instruction. Mean and standard deviations for the triplicate wells were reported for each sample.

After being cultured for 3 days, the cell samples were rinsed three times with PBS to remove non-adherent cells, and then fixed with 2.5% glutaraldehyde at 4 °C for 2 h. Thereafter, the samples were dehydrated through a series of gradient ethanol solutions of 30%, 50%, 70%, 80%, 90%, 95%, and 100%, and air-dried overnight. The morphology of cells was observed by SEM with an accelerating voltage of 15 kV after sputter coated with a 10 nm thick gold film.

RESULTS AND DISCUSSION

Loading of Dox onto MWCNTs

Dox is an anticancer drug widely used in chemotherapy due to its efficacy in fighting a wide range of cancers^[31]. Similar to other cancer drugs, the prominent cytotoxicity of Dox to normal cells or tissues is the major problem limiting its clinical applications, and hence various carrier systems have been developed to control the drug release rate and improve its bioavailability^[2, 32, 33]. MWCNTs have shown obvious advantages in loading and controlled release of Dox and other drugs^[34], since Dox can be easily loaded onto MWCNTs by π - π stacking of these aromatic molecules onto the polyaromatic surface of nanotubes, and their ultrahigh surface area permits efficient loading of multiple molecules alongside the nanotube wall^[35–38]. Moreover, the high strength and module and outstanding physical performance of MWCNTs may provide great convenience for the increased mechanical property of nanofibers by doping MWCNTs into PLGA nanofibers. Therefore, MWCNTs with carboxyl residues were chosen as the inorganic component to load Dox in this work.

The effect of pH on the loading efficiency of Dox onto MWCNTs was evaluated firstly (Fig. 1). The weight ratio between Dox and MWCNTs was set at 1:1, and the pH of the mixture was changed to 7, 8, 9, and 9.5, respectively. After 24 h of interaction between Dox and MWCNTs, the mixtures were centrifuged and the photograph was taken (Fig. 1a). With pH increasing, the red color in supernatant faded prominently, indicating that most of Dox was loaded onto the MWCNTs in a basic environment. To quantitatively evaluate the pH impact, Dox loading efficiencies at different pH were calculated (Fig. 1b). A remarkable increase of Dox loading efficiency from $(17.2 \pm 4.57)\%$ at pH = 7 to $(83.7 \pm 3.09)\%$ at pH = 9.5 was seen. This increase should be attributed to an enhanced π - π stacking interaction between Dox and MWCNTs in a basic environment, in agreement with the literature^[5]. Therefore, Dox/MWCNTs powder used for electrospinning was obtained at pH = 9.5.

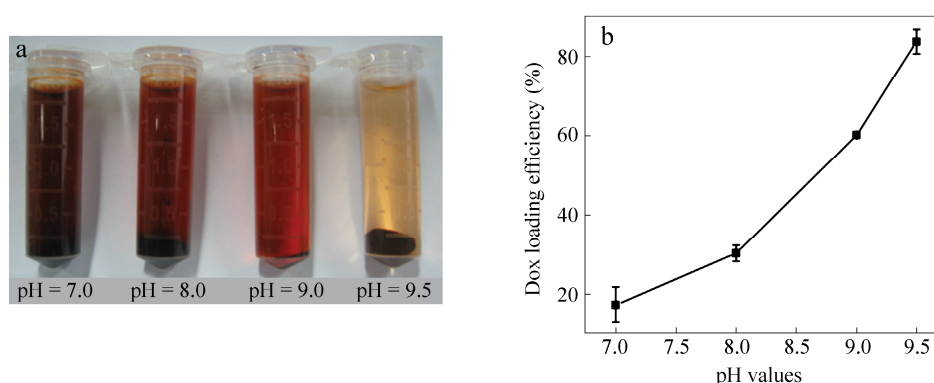


Fig. 1 (a) The photographs of the supernatants of Dox-loaded MWCNTs after centrifugation (Dox loading was performed under different pH conditions and at a mass ratio of 1:1 for Dox/MWCNTs (1 mg/mL for both).); (b) Dox loading efficiency under different pH values

Characterization and Cytocompatibility Assay of MWCNTs/PLGA Composite Nanofibrous Mats

Figure 2 shows the morphology and fiber diameter distribution histogram for the electrospun PLGA,

MWCNTs/PLGA (with 3 wt% or 5 wt% of MWCNTs) nanofibers. According to our previous study, MWCNTs was well embedded in the PLGA fiber matrix and highly oriented along the fiber axis^[29], and hence the 3-dimensional fibrous morphology of the PLGA nanofibers did not significantly change after the incorporation of MWCNTs, in agreement with the literature^[39–41]. The average diameter of the MWCNTs/PLGA composite nanofibers ((660 ± 175) nm for the sample with 3 wt% of MWCNTs, (661 ± 175) nm for the sample with 5 wt% of MWCNTs) was slightly larger than that of pure PLGA fibers ((654 ± 148) nm), which was mainly due to the incorporation of MWCNTs within the nanofibers.

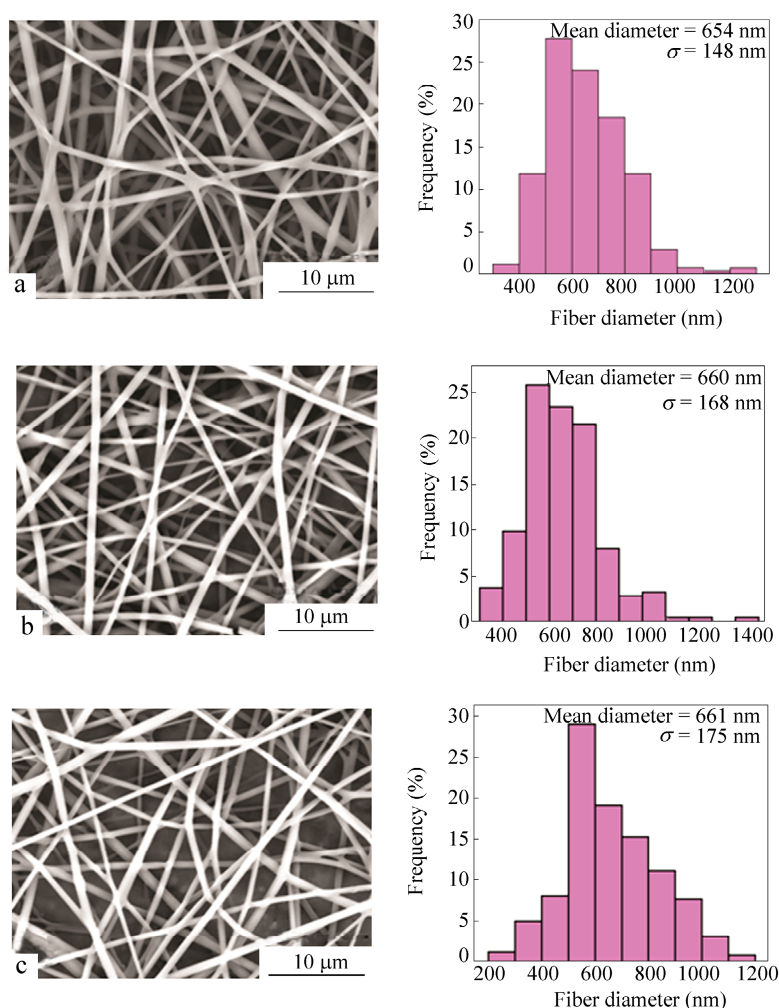


Fig. 2 SEM micrographs and fiber diameter distribution histograms of the electrospun (a) PLGA fibers, (b) MWCNTs/PLGA fibers (3 wt% of MWCNTs), and (c) MWCNTs/PLGA fibers (5 wt% of MWCNTs)

MTT cell viability assay and SEM were used to observe cell morphology and to evaluate the cytocompatibility of the MWCNTs/PLGA composite nanofibrous mats (Fig. 3). It was seen that L929 cells proliferated better on electrospun nanofibrous scaffolds than on cover slips due to the three-dimensional structure of electrospun nanofibers with interconnected pores, which could closely imitate the natural ECM and provide an appropriate microenvironment for cell attachment and migration. The viability of cells cultured onto MWCNTs/PLGA nanofibrous mats was similar to that of TCP and better than that of cover slips, suggesting the good cytocompatibility of MWCNTs/PLGA composite fibrous scaffolds.

The morphology of L929 cells cultured onto electrospun PLGA and MWCNTs/PLGA nanofibrous mats

after 3 days is shown in Fig. 4. Cells spreaded well on the electrospun nanofibrous mats and displayed a phenotypic shape, indicating that the cells penetrated in and migrated within these mats in a manner similar to native ECM. The SEM cell morphology data validated the MTT assay results, suggesting that these three-dimensional PLGA-based scaffolds were of excellent cytocompatibility to promote cell proliferation.

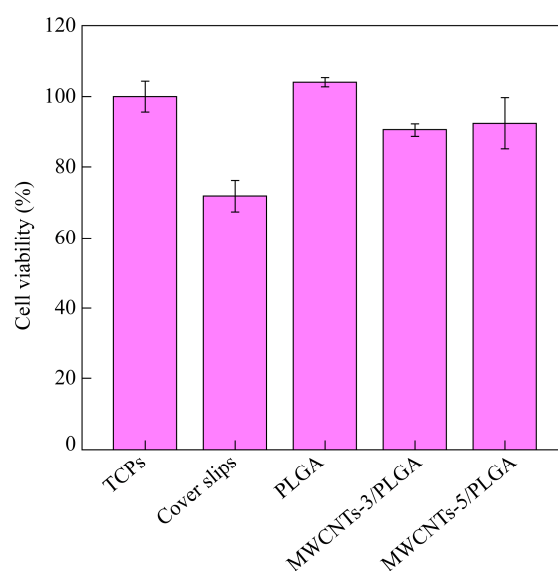


Fig. 3 Proliferation of L929 cells seeded onto the cover slips, TCPs, PLGA fibers, MWCNTs/PLGA with 3 wt% and 5 wt% of MWCNTs relative to PLGA

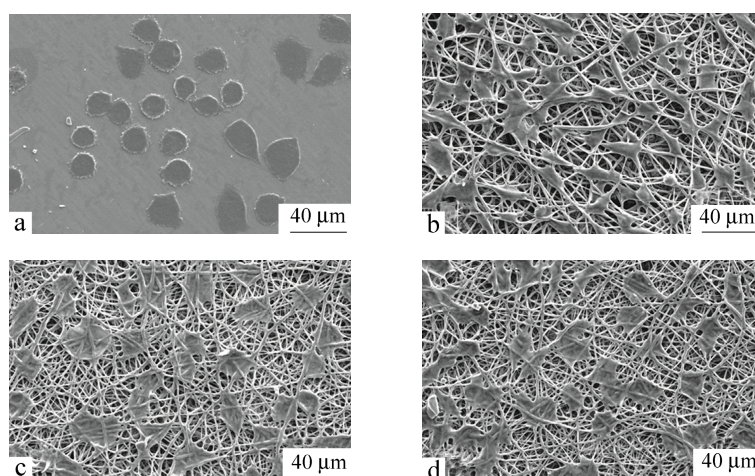


Fig. 4 SEM micrographs of fibroblasts grown on (a) cover slips, (b) electrospun PLGA fibers, (c) MWCNTs/PLGA nanofibers with 3 wt% of MWCNTs relative to PLGA, and (d) MWCNTs/PLGA nanofibers with 5 wt% of MWCNTs relative to PLGA

Characterization of Drug-loaded Composite Nanofibrous Mats

In this study, the conventional Dox-loaded PLGA nanofibers (Dox/PLGA) were also prepared by electrospinning mixture solution of Dox and PLGA. SEM micrographs and corresponding fiber diameter distribution histograms of the electrospun Dox/PLGA blend fibers and Dox/MWCNTs/PLGA medicated fibers are shown in Fig. 5. The uniform fibrous structure of PLGA fibers did not significantly change after the incorporation of Dox and Dox/MWCNTs, but the diameter of Dox/PLGA blend fibers was much smaller than that of pure PLGA fibers. It is generally known that the property of an electrospinning solution can be

significantly affected by addition of an anionic or a cationic species. Therefore, the introduction of cationic drug Dox into the electrospinning solution may cause an increase in the surface charge density of the spinning jet and result in a smaller fiber diameter. Moreover, to avoid droplets formation during the electrospinning process, the electrospinning parameter of Dox/PLGA was adjusted to a higher applied voltage, longer electrospinning distance and slower spinning speed, which may also lead to a decrease in fiber diameter. Therefore, the average diameter of DOX/PLGA nanofibers ((298 ± 92) nm) was much smaller than that of PLGA fibers ((654 ± 148) nm).

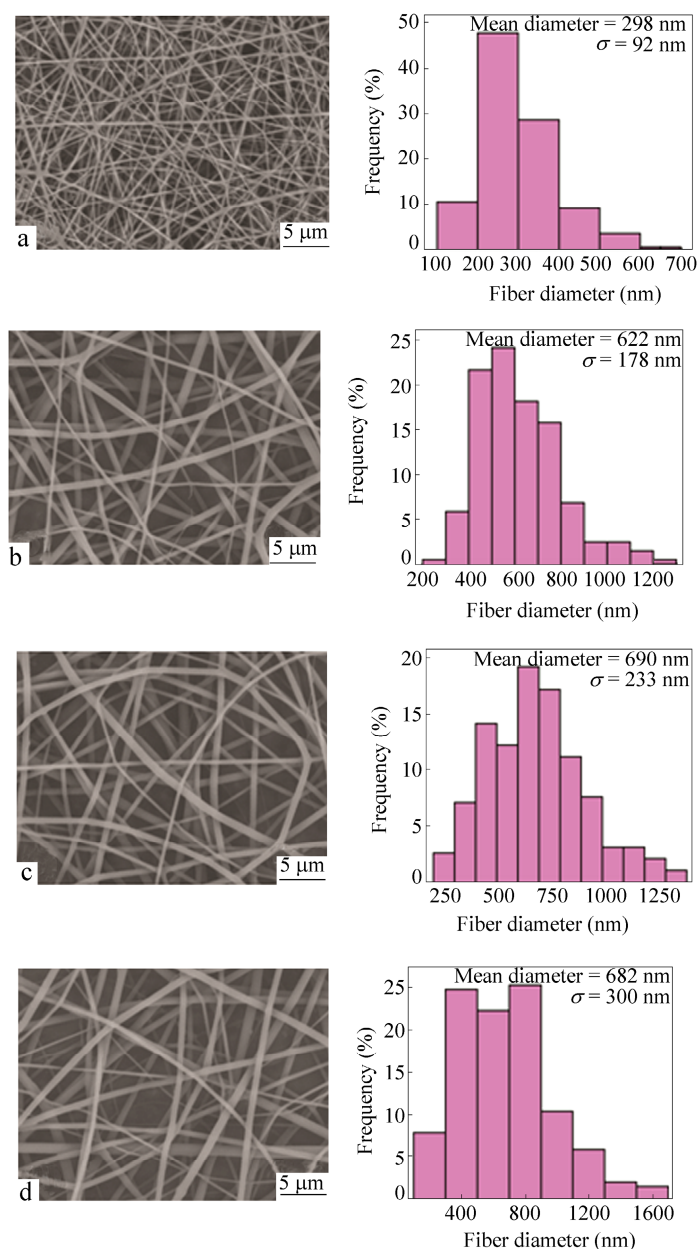


Fig. 5 SEM micrographs and corresponding fiber diameter distribution histograms of the electrospun (a) Dox/PLGA fibers with 1 wt% of Dox relative to PLGA, (b), (c) and (d) Dox/MWCNTs/PLGA mediated fibers with 1 wt%, 2 wt% and 3 wt% of Dox relative to PLGA, respectively

Compared with PLGA nanofibers, DC-1/PLGA with a lower loading amount of Dox/MWCNTs demonstrated a slightly decreased diameter ((622 ± 178) nm), while the diameters of DC-2/PLGA and DC-3/PLGA nanofibers increased to (690 ± 233) nm and (682 ± 300) nm, respectively. The changes in diameter of drug-loaded composite nanofibers mainly depend on the combination of two essential elements. On one hand, the incorporation of positively charged Dox into the electrospinning solution may significantly increase the surface charge density of the spinning jet, resulting in a decrease of fiber diameter. On the other hand, the higher concentration of MWCNTs incorporated in the composite nanofibers may cause a slight increase in the diameter of the composite nanofibers, in agreement with our previous study^[27].

Mechanical property is of paramount importance for the fabricated nanofibers to be used in tissue engineering applications. The representative strain-stress curves of electrospun PLGA, MWCNTs/PLGA, Dox/PLGA, and Dox/MWCNTs/PLGA nanofibrous mats are shown in Fig. 6, and the mean mechanical parameters are summarized in Table 1. Compared with PLGA nanofibrous mats, the breaking strength and failure strain of MWCNTs/PLGA (of 1.27 wt% MWCNTs) was improved by 30.2% and 12.3%, and its Young's modulus remained unchanged. Dox/MWCNTs/PLGA medicated fibers also showed higher breaking strength and failure strain, while their Young's modulus was lower than that of PLGA nanofibers and decreased with increase in the loaded Dox/MWCNTs. This may be caused by nonuniform distribution of Dox/MWCNTs in PLGA fibers at higher content and the ineffective load transfer from PLGA to Dox/MWCNTs. Compared with PLGA and Dox/MWCNTs/PLGA composite nanofibers, Dox/PLGA blended nanofibers displayed a slightly higher breaking strength and a much lower failure strain due to the smaller fiber diameter (Fig. 6). This suggests that the Dox/PLGA nanofibrous mats tended to have a shape distortion in practical applications. Therefore, the introduction of MWCNTs could improve the mechanical property of PLGA fibers, and Dox/MWCNTs/PLGA fibers showed a much more dimensional stability and were amenable for their applications in tissue engineering in comparison with Dox/PLGA fibers. ATR-FTIR spectroscopy was used to further characterize the composite nanofibrous mats (Fig. S1, Supporting Information), and the results revealed that the internal structure of PLGA fibers was not changed after incorporation of Dox and Dox/MWCNTs.

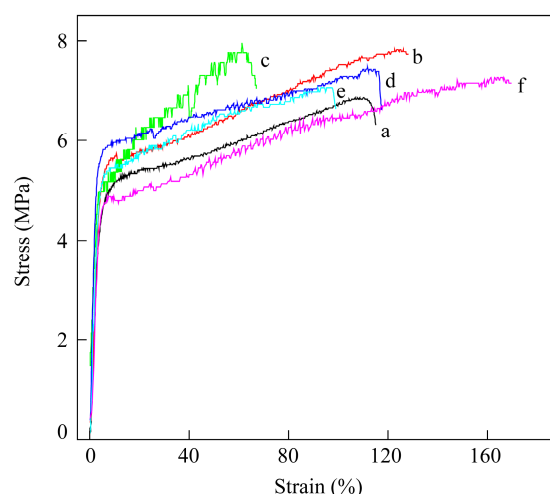


Fig. 6 Strain-stress curves of electrospun (a) PLGA, (b) MWCNTs (1.27 wt%)/PLGA, (c) Dox/PLGA (1 wt% of Dox relative to PLGA), (d) DC-1/PLGA, (e) DC-2/PLGA and (f) DC-3/PLGA nanofibrous mats

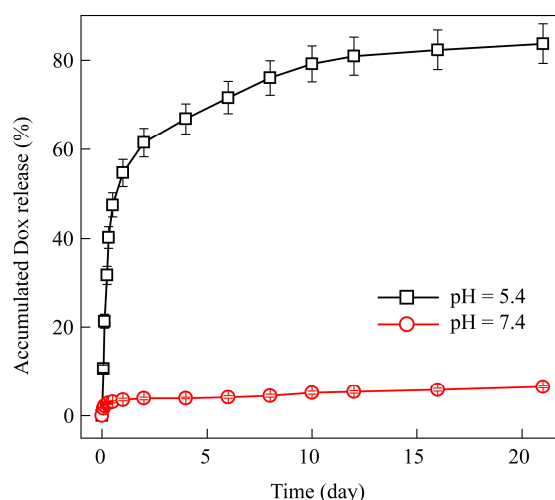
Table 1. Tensile properties of electrospun PLGA, MWCNTs/PLGA, Dox/PLGA and Dox/MWCNTs/PLGA nanofibrous mats under same processing conditions

Sample	Breaking strength (MPa)	Failure strain (%)	Young's modulus (MPa)
PLGA	6.00 ± 0.79	108.7 ± 15.45	147.7 ± 8.97
MWCNTs/PLGA	7.81 ± 0.34	122.0 ± 10.94	146.5 ± 9.07
Dox/PLGA	8.32 ± 0.32	62.4 ± 3.79	133.2 ± 4.63
DC-1/PLGA	7.05 ± 0.58	106.8 ± 2.54	142.4 ± 3.39
DC-2/PLGA	7.17 ± 0.53	141.8 ± 31.5	139.7 ± 2.81
DC-3/PLGA	6.61 ± 0.61	141.6 ± 21.8	125.5 ± 4.53

Data are representative of independent experiments and all data are given as mean ± SD ($n = 5$).

Release of Dox from the Composite Nanofibrous Mats

To exert the therapeutic functionality, the loaded Dox drug has to be released in a sustained pattern. We systemically explored the release property of Dox from the Dox/MWCNTs powder, electrospun Dox/MWCNTs/PLGA and Dox/PLGA nanofibrous mats. Firstly, the release profiles of Dox from Dox/MWCNTs powder in acetate buffer (pH = 5.4) and PBS (pH = 7.4) were evaluated (Fig. 7). In acetate buffer, Dox exhibits an obvious initial burst release and about 61.5% of the loaded Dox was released after 2 days. On the contrary, in PBS, the percentage of Dox released from the Dox/MWCNTs powder is about 3.94% after 2 days and only 6.59% even after 21 days. Therefore, the release rate of Dox at pH = 5.4 is significantly higher than that at pH = 7.4. The sensitive release of Dox in acidic environment may facilitate the specific killing of cancer cells, since the extracellular pH value in tumor tissues are slightly lower than that in normal ones. The higher release rate of Dox under acidic pH condition than under physiological condition could be due to the fact that the water-solubility of Dox is better at lower pH than at basic pH, in agreement with the literature^[19]. Therefore, in following study, the release of the medicated nanofibers was evaluated in acidic environment.

**Fig. 7** *In vitro* release profiles of Dox from Dox/MWCNTs powder at different pH values

The release profiles of electrospun Dox/PLGA with 1 wt% Dox and Dox/MWCNTs/PLGA with 1 wt%, 2 wt%, 3 wt% Dox nanofibrous mats in acetate buffer (pH = 5.4) are shown in Fig. 8. Dox/PLGA fibrous mats display a burst release within 2 days, and then a sustained release profile within 25 days with only 7.9% of Dox released. The intense electrostatic interaction between the amine group of Dox and the carboxyl group of PLGA may account for the slow release rate at this period^[42]. Another burst release appears after 25 days, and 25.56% of Dox is released after 42 days. This may be caused by the degradation of PLGA after long-term immersion in buffer medium, in agreement with the literature^[43, 44]. In contrast, Dox/MWCNTs/PLGA fibrous scaffolds display a sustained release profile during the 42 days' release. For DC-1/PLGA, DC-2/PLGA, and DC-3/PLGA

nanofibers, the release rate increases with the concentration of Dox loaded in the nanofibers, and even after 42 days, only 8.46%, 10.46%, and 11.86% of Dox is released, respectively. The sustained release property of Dox from the medicated Dox/MWCNTs/PLGA nanofibers with 1 wt%, 2 wt%, 3 wt% Dox should be due to the fact that Dox needs to be first released from MWCNTs to the PLGA matrix, and then from the PLGA matrix to the release medium, thereby significantly reducing the diffusion rate of the Dox. In addition, according to the study performed by Xu *et al.*^[43], the incorporation of MWCNTs within PLGA nanofibers is able to slow down the degradation rate of PLGA nanofibers. Taken together, the incorporation of MWCNTs seems to not only enlarge the Dox diffusion distance, but also slow down the degradation rate of the PLGA nanofibers, thereby allowing for sustained release of Dox. Overall, our results suggest that both the PLGA nanofibers and the MWCNTs can be used to hinder the release of Dox to the solution. More importantly, after being incorporated within the nanofibrous mats, the Dox/MWCNTs could be formulated as a drug-containing scaffolding material for both tissue engineering and pharmaceutical applications. In the following work, *in vitro* and *in vivo* experiments of the medicated Dox/MWCNTs/PLGA nanofibrous mats will be performed to evaluate their antitumor activity.

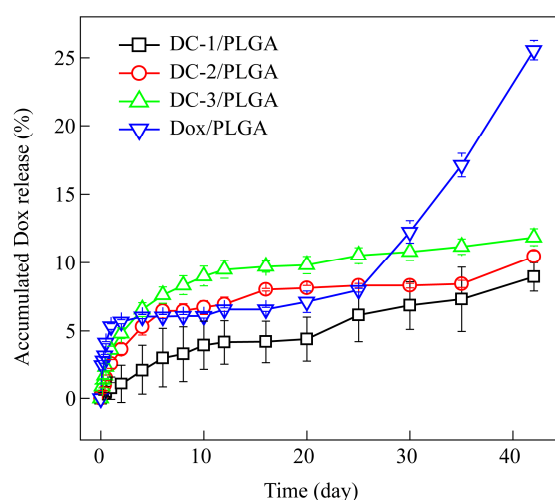


Fig. 8 *In vitro* release profiles of Dox from electrospun Dox (1 wt%)/PLGA blend nanofibers, DC-1/PLGA, DC-2/PLGA, and DC-3/PLGA nanofibrous mats (The samples were incubated in acetate buffer (pH = 5.4) at 37 °C.)

CONCLUSIONS

In summary, we developed an electrospun composite MWCNTs/PLGA nanofiber-based drug delivery system for efficient loading and sustained release of antitumor drug Dox. The incorporation of Dox-loaded MWCNTs in PLGA nanofibers does not change the uniform fibrous structure of the PLGA nanofibers, instead significantly improves their mechanical properties. Using the double containers of MWCNTs and PLGA fibers, Dox can be released from the MWCNTs/PLGA nanofibrous mats in a sustained manner over 42 days, appreciably weakening the initial burst release characteristics of the conventional electrospun polymer fiber-based delivery system. Therefore, the developed Dox/MWCNTs/PLGA composite nanofibrous mats may have potential applications in post-operative local chemotherapy and tissue engineering.

Supporting Information Available: Additional FTIR characterization data of PLGA, Dox/PLGA, and Dox/MWCNTs/PLGA nanofibrous mats.

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