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Research Paper

Electrospun Micro- and Nanofibers for Sustained Delivery of Paclitaxel to Treat C6 Glioma *in Vitro*

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Purpose. The present study aims to develop electrospun PLGA-based micro- and nanofibers as implants for the sustained delivery of anticancer drug to treat C6 glioma *in vitro*.
Methods. PLGA and an anticancer drug—paclitaxel-loaded PLGA micro- and nanofibers were fabricated by electrospinning and the key processing parameters were investigated. The physical and chemical properties of the micro- and nanofibers were characterized by various state-of-the-art techniques, such as scanning electron microscope and field emission scanning electron microscope for morphology, X-ray photoelectron spectroscopy for surface chemistry, gel permeation chromatogram for molecular weight measurements and differential scanning calorimeter for drug physical status. The encapsulation efficiency and *in vitro* release profile were measured by high performance liquid chromatography. In addition, the cytotoxicity of paclitaxel-loaded PLGA nanofibers was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide MTT) assay on C6 glioma cell lines.
Results. PLGA fibers with diameters of around several tens nanometers to 10 μm were successfully obtained by electrospinning. Ultrafine fibers of around 30 nm were achieved after addition of organic salts to dilute polymer solution. The encapsulation efficiency for paclitaxel-loaded PLGA micro- and nanofibers was more than 90%. DSC results suggest that the drug was in the solid solution state in the polymeric micro- and nanofibers. *In vitro* release profiles suggest that paclitaxel sustained release was achieved for more than 60 days. Cytotoxicity test results suggest that IC50 value of paclitaxel-loaded PLGA nanofibers (36 μg/ml, calculated based on the amount of paclitaxel) is comparable to the commercial paclitaxel formulation-Taxol®.
Conclusions. Electrospun paclitaxel-loaded biodegradable micro- and nanofibers may be promising for the treatment of brain tumour as alternative drug delivery devices.

KEY WORDS: electrospinning; glioma; microfibers; nanofibers; paclitaxel; PLGA.

INTRODUCTION

Polymeric fibers have wide applications in research areas such as composites, protective clothing, catalysis, electronics, biomedical applications, filtration, agricultures and others (1,2). With respect to biomedical applications, electrospun fibers are mainly used in tissue engineering, implants, biosensors, membranes, wound dressing, and controlled drug delivery systems (3,4). Some of these investigations focus on using nanofibers as tissue scaffolds or encapsulating growth factors in nanofibers as bio-functional scaffolds for biomedical applications (5–7). Chu *et al*. investigated biodegradable and/or bioabsorbable fibrous articles for medical applications (4). Kenawy *et al*. reported electrospun nanofibers of poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend to encapsulate Tetracycline hydrochloride for the treatment of periodontal disease (8). Luu *et al*. utilized electrospinning to fabricate synthetic polymer/DNA composite scaffolds for therapeutic application in gene delivery for tissue engineering. They demonstrated that DNA released directly from these electrospun scaffolds was intact, capable of cellular transfection, and successfully encoded the protein β-galactosidase (9). Antibiotics encapsulated in fibrous matrix as wound dressing has been reported by Kim *et al*. (10). In a separate study, aqueous domain was encapsulated in fibers by the electrospinning technique and the results suggested that under proper conditions it should be possible to trap living cells within the reservoirs (11). Living cells can also adhere to the electrospun fibrous matrix and these cells can be released in a controlled manner (12). Recently, doxorubicin hydrochloride was encapsulated in PEG-PLLA nanofibers by electrospinning of an emulsion of aqueous Doxorubicin solution in PEG-PLLA/chloroform solution (13). More recently, there has been increasing interests on the encapsulation of paclitaxel in PLA fibers (14,15). However, the fibre diameter range was limited in the size range from 300 nm to 1.2 μm and the physical and chemical characterization was incomplete.

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Several brain drug delivery systems were reported in the literature, including the forms of nanoparticles, catheters, manipulation of blood brain barrier, and Gliadel® wafer. Preclinical studies showed that rats with glioblastomas had longer survival time when their tumors were treated with doxorubicin bound to polysorbate-coated nanoparticles (with diameters of 200–400 nm) than other formulations of doxorubicin. However, these types of nanoparticles have not yet been used clinically (16). Catheter systems have been in clinical use for many years and these devices are limited by mechanical failure, obstruction by tissue debris or clot, and infection (17). Manipulation of natural permeability properties of the BBB has just been shown to modestly improve survival in patients (17). Commercial implants like the Gliadel® Wafer delivering BCNU (Carmustine) has demonstrated some success in improving patient survival rates to treat brain tumours (18). However, a limitation of Gliadel® treatment is the resistance of many brain tumours to BCNU because AGT, a DNA-repair protein found in the majority of brain tumours, is responsible for much of the resistance and it is only be useful in a very limited number of patients (19). These results highlighted the possibility of using biodegradable polymers as a vehicle for other chemotherapeutic drugs.

Paclitaxel is a very good antineoplastic agent against many types of cancers such as breast cancer and ovarian cancer and its terminal half-life was found to be in the range of 1.3–8.6 h (20). Due to the hydrophobic nature, the current commercial formulation of paclitaxel-Taxol® which consists of 1:1 blend of Cremophor EL and ethanol has many side effects including hypersensitivity reactions, nephrotoxicity and neurotoxicity (20). Also, paclitaxel has shown cytotoxicity against Glioma *in vitro* up to the scale of nanomolar (21). However, since paclitaxel is a possible substrate of the multidrug resistance protein p-gp, it is prevented from entering the brain (22) and penetrating intact blood-brain barrier poorly (23,24). Biodegradable polymer implant was developed to deliver paclitaxel against experimental malignant glioma and found to be efficient in a rat model (25). Paclitaxel-loaded micro- and nanofibers could be an alternative approach and a promising implant to treat brain tumour. For clinical applications, extra surgeries will be required to implant micro- or nanofiber flat mats which can be either fabricated or modified to almost any size and this approach represents an attractive form for local delivery applications with a variety of shapes (e.g., tubes) constructed using different target geometries (8). In the present study, the main objective is to obtain controllable diameter of PLGA-based fibres and to encapsulate paclitaxel in electrospun fibers as topical drug delivery devices to treat C6 glioma *in vitro*. Since paclitaxel is capable of radiosensitizing glioma *in vitro* (26), the implants developed in this study could also be combined with radiotherapy to enhance the drug efficacy.

MATERIALS AND METHODS

Paclitaxel used in the present study was a generous gift from Bristol Meyer Squibb Company (Princeton, NJ, USA). Poly (D, L-lactic-co-glycolic acid) (PLGA) (MEDI SORB® Low I.V.) with L: G molar ratio of 50:50 was purchased from Lakeshore biomaterials (Cincinnati, OH, USA). Tetrabutylammonium tetraphenylborate (TATPB) of electrochemical grade was bought from Fluka Chemie (Switzerland). Fluorescein diacetate (FDA) and phosphate buffered saline (PBS) used for *in vitro* release study was bought from Sigma Aldrich (St. Louis, MO, USA) containing 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.4. Dicholoromethane (DCM), Acetonitrile and Tetrahydrofuran (THF) of HPLC/Spectro grade were acquired from Tedia Company (Farifield, OH, USA). All other materials and reagents used were of analytical grade.

Fibers Fabrication by Electrospinning

The electrospinning experimental setup is shown in Fig. 1a. PLGA with and without different amount of organic salt TATPB was dissolved in DCM at concentrations between 2 and 30% (w/v). Polymer solution was pumped at a predetermined rate using a syringe pump, forming a bead of solution at the tip of syringe. A high voltage difference (5–12 kV) was applied between the nozzle (a 21-gauge or 27-gauge needle with diameter of 0.91 or 0.34 mm) and a grounded collection target.

Morphology

The morphology of fibers was observed using scanning electron microscopy (SEM) and field emission scanning electron microscopy (FSEM). SEM (Jeol JSM 5600LV, Tokyo, Japan) and FSEM (Jeol JSM, Tokyo, Japan) require an ion coating with platinum by a sputter coater (JFC-1300, Jeol, Tokyo, Japan) for 40 seconds in a vacuum at a current intensity of 40 mA after preparing the sample on metallic studs with double-sided conductive tape. The accelerating voltage ranged

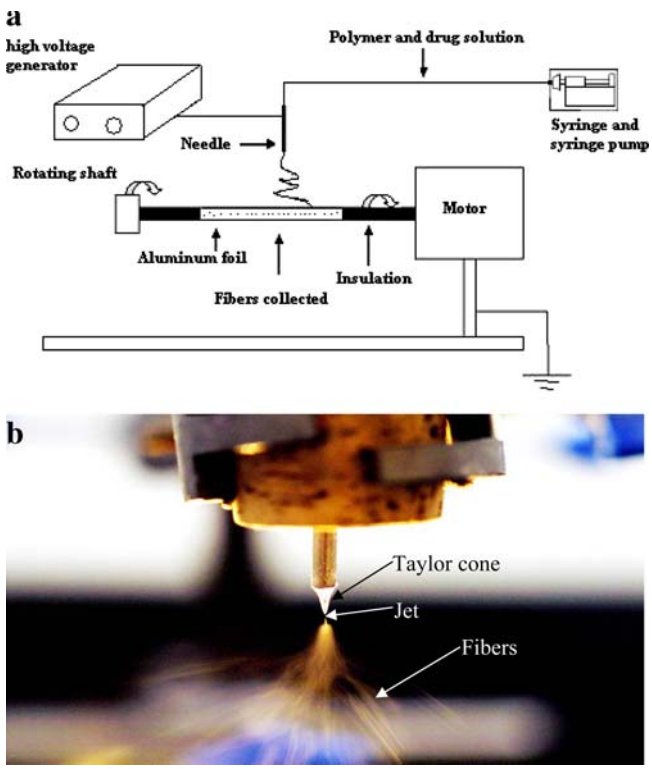

Fig. 1. (a) Schematic of electrospinning setup; (b) representative image of electrospinning jet with the exposure time of 10 ms.

from 5–15 kV during scanning. The pore size and fibre diameters were determined by the SEM software Smile View (version 2.0, JEOL) ($n > 50$).

Physical State of the Drug in the Polymer Matrix

The samples before and after *in vitro* release were first freeze dried before any analysis. Around 8 mg of sample was sealed in standard aluminium pans with lids. The sample was purged with pure dry nitrogen at a flow rate of 20 ml/min. The temperature ramp speed was set at 10°C/min and the heat flow was recorded from 0 to 250°C. Annealing tests were performed by keeping the samples at 60°C for 3 days. Then the samples were cooled down by natural convection with ambient air to room temperature. After the first heating run, temperature was cooled down and then the second heating run was performed. Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument (DSC 822e Mettler Toledo, Greifensee Switzerland).

X-ray Photoelectron Spectroscopy (XPS)

The surface chemistry of the micro- and nanofibers was determined by AXIS His X-ray photoelectron spectroscopy (Kratos Analytical Inc., NY) using the curve fitting software provided by the manufacturer. For all samples, survey spectrum was recorded over a binding energy range of 0–1,100 eV using a pass energy of 80 eV. In all cases, the survey spectra recorded the presence of oxygen (O1s 533 eV), Carbon (C1s 285 eV) and nitrogen (N1s399 eV) at the surface.

Gel Permeation Chromatography (GPC)

The PLGA molecular weight distribution was determined using GPC equipped with a refractive index detector (Waters, Model 1515, Mildford, Massachusetts). The samples were dissolved in THF and eluted at a flow rate of 1 ml/min. Polystyrene standards (MW from 1.31×10^3 to 1.97×10^5 Da) (Polysciences, Warrington, Pennsylvania) were used to obtain a primary calibration curve.

Encapsulation Efficiency and *in Vitro* Release

To determine encapsulation efficiency, 3 mg of paclitaxel-loaded PLGA micro/nanofibers were dissolved in 1 ml DCM. Subsequently, 9 ml of mobile phase (acetonitrile:water = 1:1) was added to the solution. A nitrogen stream was introduced to evaporate DCM at room temperature until a clear solution was obtained. The resulting solution was then filtered and analyzed by high performance liquid chromatography (HPLC) under the condition mentioned below.

Around 10 mg of micro- and nanofiber fabrics were cut and the samples were stored in 10 ml of PBS (pH 7.4)

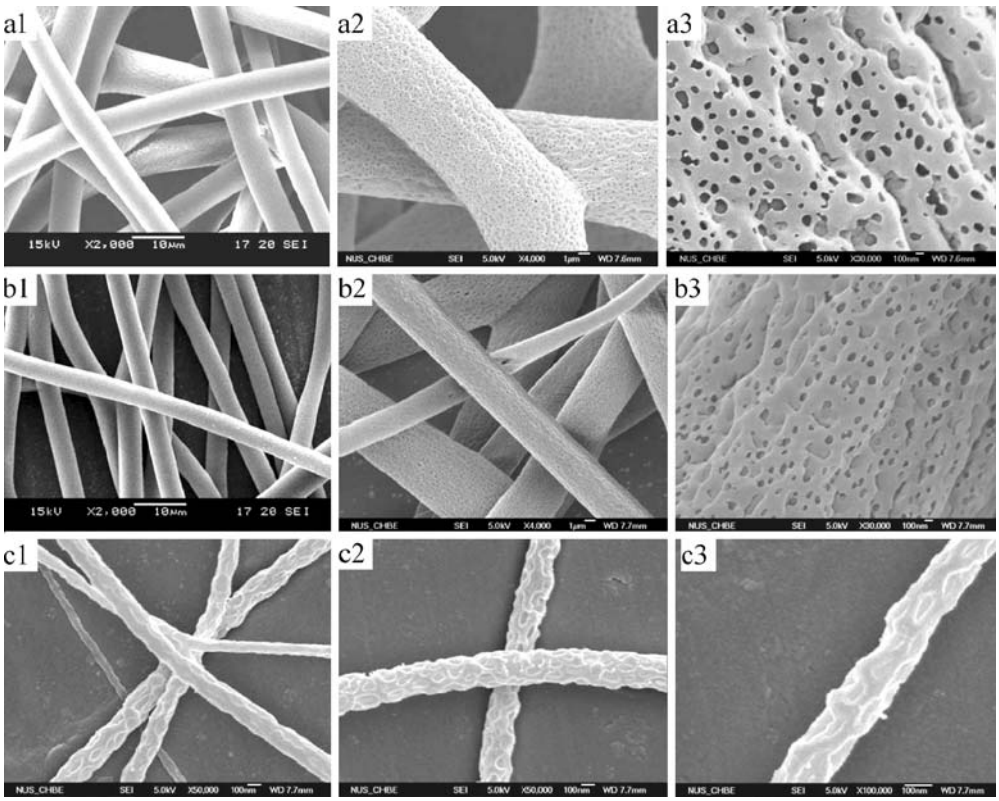

Fig. 2. SEM and FSEM images of PLGA fibers. [30% PLGA in DCM (a1) 10 ml/h (8 ± 0.4 μm); (a2), (a3) high magnification of a1 (mean pore size 125 ± 33 nm); (b1) 3 ml/h (3 ± 0.2 μm); (b2), (b3) high magnification of b1 (mean pore size 82 ± 21 nm); (c1) 10% PLGA in DCM 1 ml/h (300 ± 20 nm); (c2), (c3) high magnification of c1 (mean pore size 50 ± 9 nm)]. The diameter for each sample and pore size were measured and averaged by a computer program SMILE VIEW 2.0 ($n \geq 50$).

containing 1% of Tween 80 to enhance the solubility of paclitaxel. The buffer solution was kept at constant temperature of 37°C. At given time intervals, three tubes of each formulation of samples were withdrawn and centrifuged at 11,500 rpm for 10 min. The precipitated samples were taken and resuspended in 10 ml fresh release medium to keep a complete sink condition and placed back to the shaker. The supernatant solution was retained for HPLC analysis. Paclitaxel in the release medium was first extracted with 1 ml of DCM. A mixture of acetonitrile and water (50:50 v/v) was added to the extracted paclitaxel after the DCM had evaporated. The resulting solution was analyzed using HPLC, in which a C-18 Column was used and the mobile phase was delivered at a rate of 1 ml/min. One hundred microliters of sample was injected by an auto-sampler and the column effluent was detected at 227 nm using an ultra violet (UV) detector. The detection data were corrected according to the extraction efficiency.

Cell Culture

C6 glioma cells (courtesy, Singapore General Hospital, passages 6 to 10) were grown and routinely maintained at 37°C in 75 cm^2 culture flasks, in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum, 1% penicillin-streptomycin in atmosphere of 5% CO_2 and 90% relative humidity. The cells were harvested with Trypsin-EDTA. Medium was changed every other day.

Confocal Laser Scanning Microscopy (CLSM)

Cells were cultured in the 75 cm^2 culture flask. After confluence, 4×10^4 of cells per well were transferred to the 24 well plates. One column was for control with only cover slips. Another column was incubated with cover slips deposited by blank PLGA nanofibers. All other columns were incubated with cover slips deposited by different

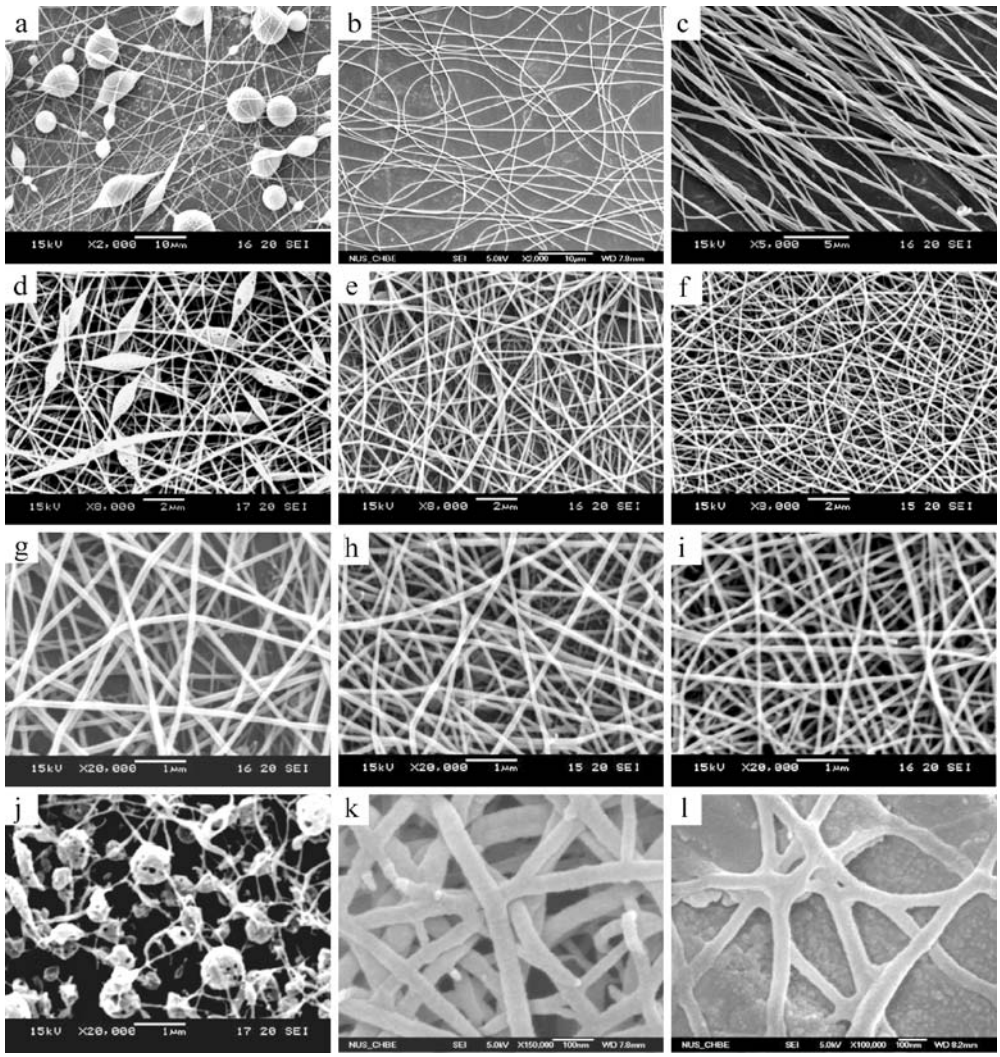

Fig. 3. SEM and FSEM images of PLGA fibers under different polymer concentrations. (a) 15%; (b) 15%, 1 mM organic salt; (c) 10%, 5 mM, nozzle 21 Gauge; (d) 5%; (e) 5%, 1 mM organic salt; (f) 5% 5 mM organic salt; (g) enlargement of e (110 ± 16 nm); (h) enlargement of f (100 ± 14 nm); (i) 5%, 15 mM organic salt (82 ± 11 nm); (j) 2%; (k) 2%, 10 mM organic salt (45 ± 7 nm); (l) 2%, 15 mM organic salt (31 ± 5 nm), nozzle 29 Gauge. The diameter for each sample was measured and averaged by a computer program SMILE VIEW 2.0 ($n \geq 50$).

Table I. Characterization of Paclitaxel-Loaded Fibers

Samples	Fibers Mean Diameter†	Drug Loading* (%)	Encapsulation Efficiency* (%)
PLGA MF (s1)	2.5 ± 0.32 μm	9.9 ± 0.1	99.0 ± 1.0
PLGA NF (s2)	770 ± 13 nm	9.2 ± 0.03	92.0 ± 0.3

† The diameter for each sample was measured and averaged by a computer program SMILE VIEW 2.0 ($n \geq 50$).
*Encapsulation efficiency and drug loading for each sample were the averages of triplicate samples.

amounts of paclitaxel-loaded PLGA nanofibers. Blank and paclitaxel-loaded PLGA nanofibers were deposited on the round glass slides and decontaminated under UV light for 6 h before incubation with C6 glioma cells. After 72-h incubation, the cells were stained with Fluorescein Diacetate (FDA) following the standard procedures. Subsequently, cover slips were taken out and samples were observed by confocal laser scanning microscopy (Zeiss LSM510).

Cytotoxicity Test

Paclitaxel-loaded PLGA nanofibers were deposited on the round glass slides and decontaminated under UV light for 6 h before cytotoxicity test. Cytotoxicity of paclitaxel-loaded PLGA nanofibers was determined against C6 glioma cells. The cell viability was determined by a microplate reader (GENios, Groedig, Austria). Cells were transferred to a 24-well plate first to ensure 4×10^4 cells per well. Medium was changed every other day. The plate was incubated for 72 h. One row of 24-well plates was used as control without adding nanofibers. One row was administered by blank PLGA nanofibers. Other rows were administered by paclitaxel-loaded PLGA nanofibers with different concentrations. Thirty microliters of MTT assay and 270 μl of medium were then added to the wells. After incubation for around 3–4 h, solution was removed, thus leaving the precipitate behind. One hundred microliters Isopropanol or DMSO was then added to the wells before the plate was observed using microplate reader. Cell viability was determined by the ratio of absorbance of test cells and absorbance of control cells at wavelength of 560 nm, where absorbance of test cells and absorbance of control cells represent the amount of formazan determined for cells treated with different formulations and for control cells (non-treated), respectively.

RESULTS AND DISCUSSION

Electrospun Fibers Fabrication

Image of electrospinning jet with an exposure time of 10 ms was shown in Fig. 1b. The effects of key processing parameters on PLGA-based fibers fabricated by electrospinning such as polymer concentrations, conductivities, polymer solution flow rates etc. were investigated. The diameters from around 30 nm to 10μm of PLGA-based fibers could be fine tuned by adjusting the processing parameters. The diameter of PLGA fibers decreased with decreasing polymer solution flow rate and polymer concentration as shown in Fig. 2a1, b1 and c1. The nanopores on the surface of PLGA microfibers were formed as shown in Fig. 2a2, a3, b2 and b3. The pore structure was also formed on the surface of PLGA nanofibers as shown in Fig. 2c2 and c3. This kind of surface structure could be due to rapid evaporation of organic solvent from the fibers. A similar phenomenon was also observed by previous reports on other types of fibers (27–29). The addition of organic salts (TATPB) in the polymer solution can reduce the beads formation as shown in Fig. 3. Ultrafine PLGA nanofibers with diameter of 30 nm could be obtained using a 2% polymer solution with a TATPB concentration of 15 mM. A similar cationic surfactant employed to remove the beads on the polystyrene fibers was reported earlier by Tong *et al*. (30).

The characterization of typical samples obtained in the present study is shown in Table I. SEM images of typical samples are shown in Fig. 4. Paclitaxel-loaded PLGA micro- and nanofibers were deposited to form non-woven fabrics as shown in Fig. 4S1 and S2. The surfaces of the non-woven fabrics were shown in Fig. 4S1a and S2a, respectively. The mean diameters of paclitaxel-loaded PLGA micro- and nanofibers were around 2.5 μm and 770 nm. The drug loadings for paclitaxel-loaded PLGA micro- and nanofibers were 9.9 and 9.2%, respectively. The encapsulation efficiency was more than 90%. Figure 4S1b and S2b showed the SEM images of 10% paclitaxel-loaded PLGA micro- and nanofibers after 61-day release. The morphology of fibers was significantly different between the samples before and after the release. Most of the fibers were broken and melting pieces were formed after the degradation for 61 days.

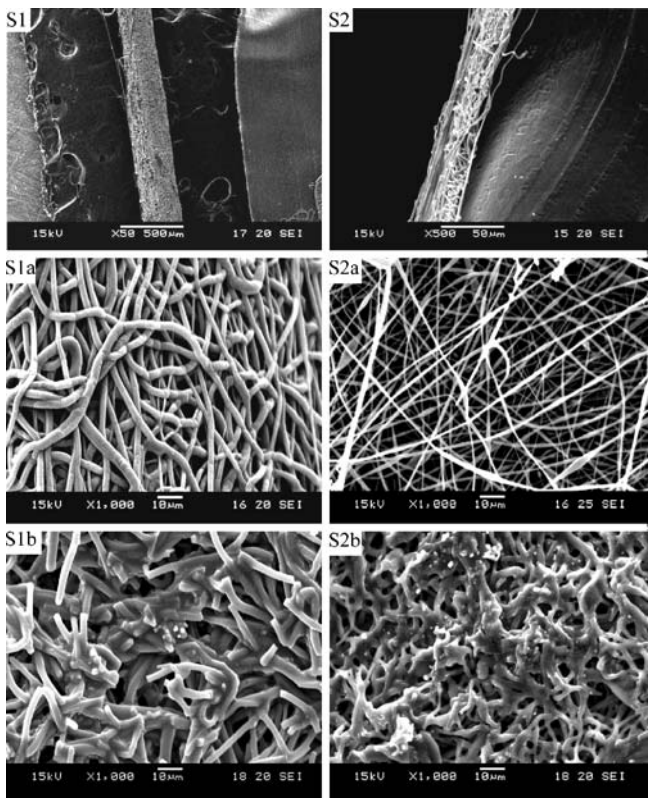

Fig. 4. SEM images of different samples. (s1) paclitaxel-loaded PLGA microfiber film; (s2) paclitaxel-loaded PLGA nanofiber film; (s1a) s1 before release; (s1b) s1 after 61-day release; (s2a) s2 before release; (s2b) s2 after 61-day release.

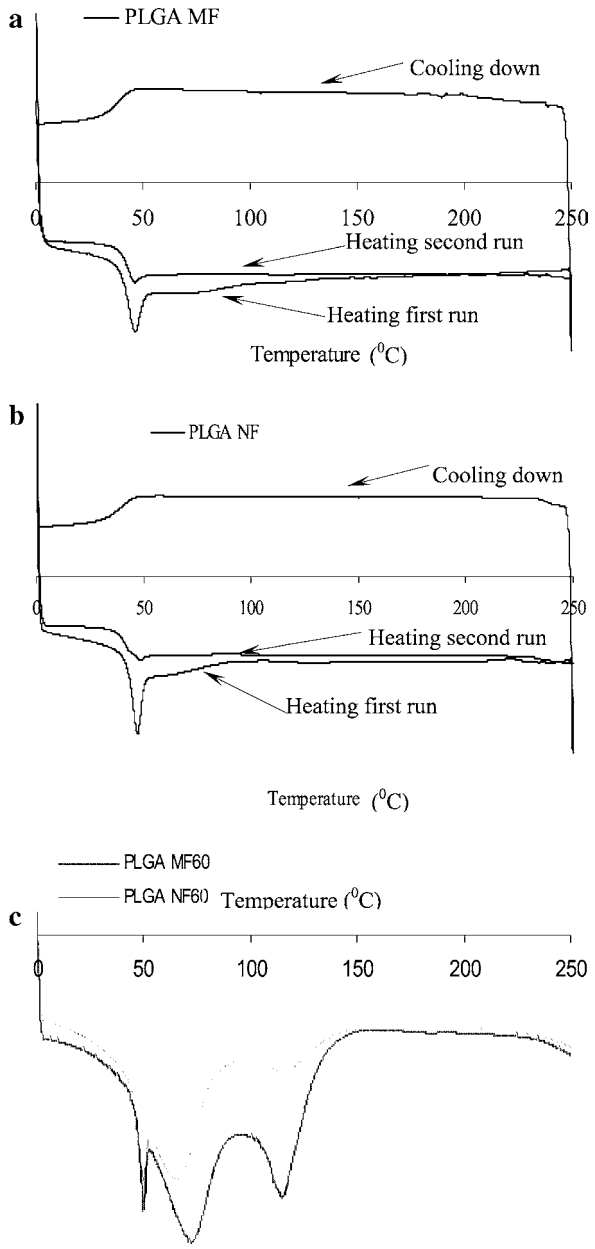

Fig. 5. DSC thermogram of different samples. (a) s1 before release; (b) s2 before release; (c) s1 and s2 after 60-day release.

Physical Characterization of Drug in Polymer Matrix

Figure 5 shows the DSC thermogram analysis to provide qualitative and quantitative information about the physical status of the drug in the micro- and nanofibers. The drug loading of the samples in DSC experiment was around 10%. All pure materials gave the peak relevant to the phase transition temperatures (T_g or T_m). The pure paclitaxel showed an endothermic peak of melting at 223.0°C. The glass transition temperature of PLGA (50:50) was shown at 45°C. There was no peak observed at the temperature range of 150–250°C for all samples. The DSC measurements did not detect any crystalline drug material in the micro- and nanofibers samples as shown in Fig. 5a and b. After annealing at 60°C for 3 days, no melting endotherm of the drug was detected. It could thus be concluded that the paclitaxel formulated in the samples was in a solid solution state in the polymer matrix after fabrication (31). This finding agreed very well with the general results of electrospinning and spray-drying (32,33). Moreover, the glass transition temperatures of the polymers employed in paclitaxel-loaded PLGA micro- and nanofibers were not obviously influenced by the procedure (data not shown). Figure 5c showed the DSC thermogram of paclitaxel-loaded PLGA micro- and nanofibers after 61-day release. The double T_g values and crystalline melting endotherm was due to two different polymer domains and oligomer crystallization effect during the degradation, respectively (34). The result also suggests that no crystalline drug in the samples after 61-day release was detected and the drug left inside fibres still maintained the state of amorphous or disordered-crystalline phase of a molecular dispersion or a solid solution.

X-ray Photoelectron Spectroscopy (XPS)

The surface chemistry of the biodegradable polymeric fibers was analyzed by X-ray photoelectron spectroscopy (XPS) to detect several nanometres depth of polymeric materials. Figure 6 shows the XPS spectroscopy of N1s and C1s of different samples. N1s spectroscopy suggests that marginal amount of nitrogen was detected in some samples.

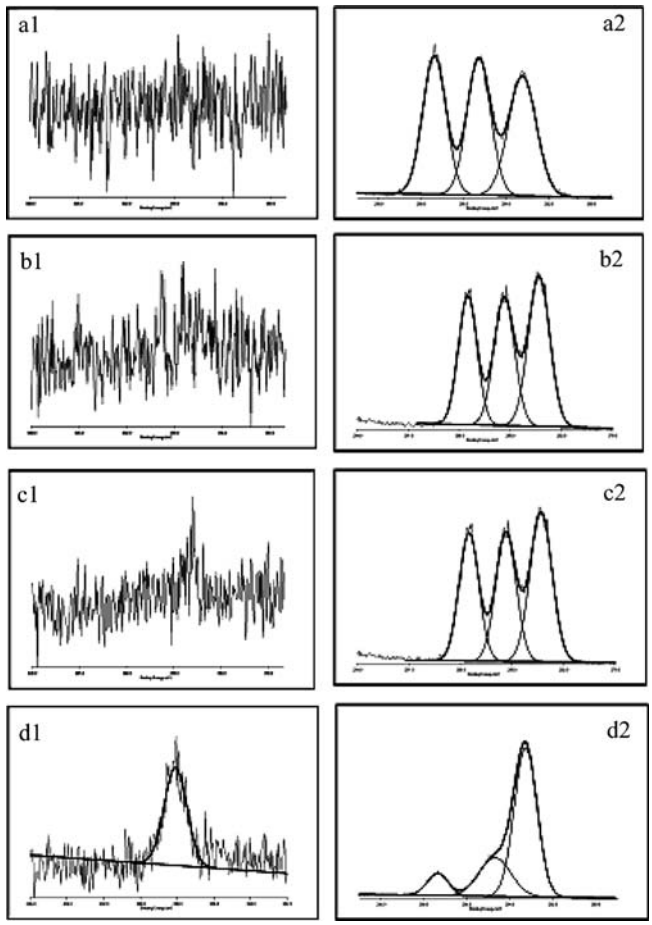

Fig. 6. N1s and C1s spectroscopy of samples. [(a1), (a2) Blank PLGA nanofibers; (b1), (b2) s1; (c1), (c2) s2; (d1), (d2) pure paclitaxel].

Table II. Surface Chemistry of Various Samples Analyzed by XPS

	XPS Elemental Ratio (%)			XPS C1s Envelope Ratio (%)		
Sample	C	N	O	C–C/ C–H	C–OH (R)	O–C=O
Paclitaxel	79.3	1.4	19.3	66.8	24.3	8.9
Blank PLGA NF	58.62	0.0	41.38	33.9	33.0	33.1
PLGA MF	60.89	0.03	39.08	39.0	31.8	29.2
PLGA NF	60.42	0.0	39.58	38.9	31.2	29.9

Blank PLGA NF PLGA nanofibers, *PLGA MF* s1, *PLGA NF* s2.

The variation of C1s spectroscopy may be due to the existence of paclitaxel on the surface layer of samples. The quantitative results were summarized in Table II, which suggests that very little amount of paclitaxel existed on the surface layer of biodegradable polymeric fibers. This could be due to the electrospinning process which employs electric energy to an organic solution of polymer material which was dispersed with the drug. It is possible for the hydrophobic drug to be present on the surface layer of the fibers that was near the hydrophobic solvent environment due to evaporation process.

Gel Permeation Chromatogram (GPC)

The gel permeation chromatograms of the degraded PLGA fibers suggest that the degradation proceeded heterogeneously as shown in Fig. 7. Also, on day 61 a secondary peak was detected at 30 min in addition to the main peak at 26 min. This indicates the presence of both fast and slow degrading domains corresponding to the inner and surface fibrous meshes. Based on the calibration data of polystyrene standards, the molecular weight at the retention time of 30 min corresponding to molecular weights was lower than 1.31×10^3 Da.

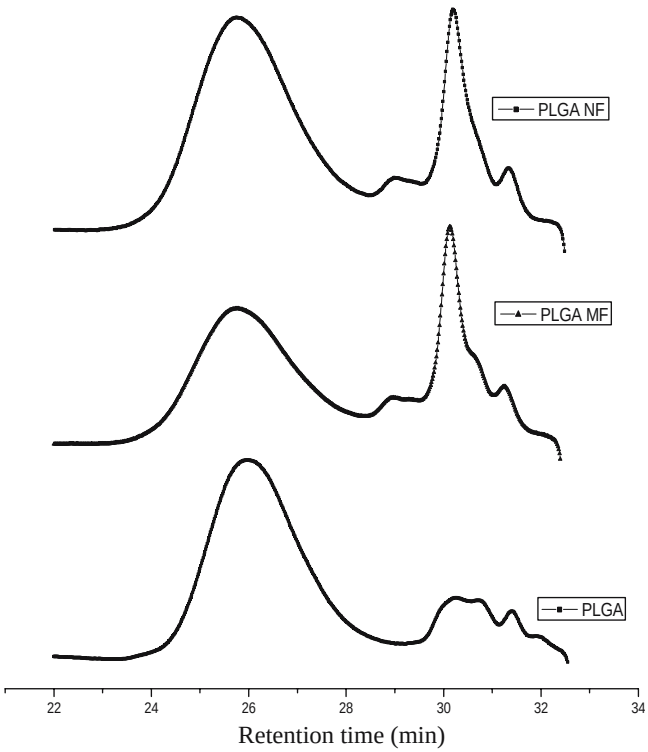

Fig. 7. Gel permeation chromatograms of PLGA fibers showing the changes in retention time after degradation. PLGA: PLGA fibers before degradation; PLGA MF: s1 degradation after 61 days; PLGA NF: s2 degradation after 61 days.

In Vitro Release Profiles

Gliadel® wafers were reported to release carmustine *in vitro* and *in vivo* over a period of approximately 5 days in previous studies (35,36): about 80% of paclitaxel was released from 20% paclitaxel-loaded PCPP-SA (20:80) polymer discs after around 37 days and 20% of paclitaxel was released after the first 2 days *in vitro*. These polymer discs were found to extend the median survival of rats bearing intracranial tumors 3.1-fold as compared to the controls (25). These data sets supported the idea that paclitaxel may be a better candidate than BCNU for sustained interstitial chemothera-

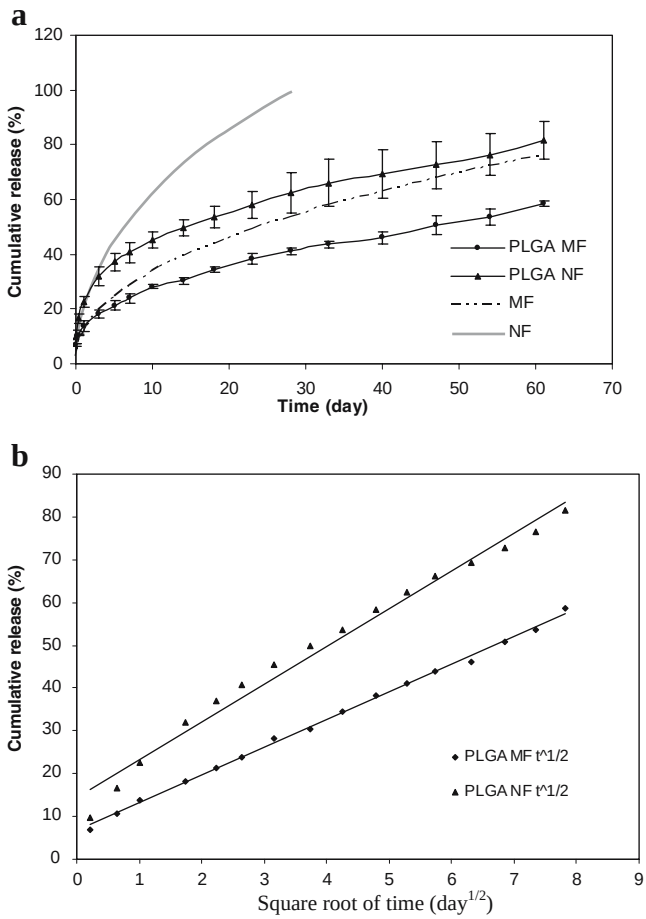

Fig. 8. (a) *In vitro* release profiles of representative samples. (●: s1; ▲: s2; MF: $k = 0.119$, $n = 0.45$ Fickian diffusion from monodispersed cylinders; NF: $k = 0.223$, $n = 0.45$ Fickian diffusion from monodispersed cylinders). Each data point represents the average of $n = 3$ samples, *error bars* represent standard deviations. (b) Cumulative release of paclitaxel as a function of the square root of time; the *solid line* is a linear fit to the data points.

py in view of the actual release profiles. However, neurotoxicity was observed due to high drug concentrations distributed throughout the rat brain (25). In view of this potential neurotoxicity, a lower paclitaxel loading (10%) was used in the present study to examine the amount of paclitaxel released from PLGA micro- and nanofibers.

Figure 8a and b showed the *in vitro* release profiles of paclitaxel-loaded PLGA micro- and nanofibers as a function of time and the square root of time, respectively. From XPS results, diffusion of little amount of paclitaxel on or nearby the surface layer of fibers could most likely contribute to the initial burst release. The release rate of paclitaxel-loaded PLGA nanofibers was faster than PLGA microfibers. Around 13 and 22% of the total amount of drugs was released in 24 h. Also, the sustained release of paclitaxel could last for more than 2 months and demonstrated a nearly first order release kinetics as shown in Fig. 8b. It was observed that the amount of paclitaxel released from PLGA micro- and nanofibers was nearly 60 and 80% of the total amount of drug in the fibers, respectively. Therefore, better sustained release properties of paclitaxel can be achieved as compared to the previously reported PCPP: SA (20:80) discs. When nanofibers are used as polymer implants, effective treatment of brain tumour without neurotoxicity could be achieved with longer exposure time and lower concentration of the drug (25). Furthermore, the prescribed time window agreed well with the period for polymer degradation. A one-dimensional drug diffusion model under perfect sink con-

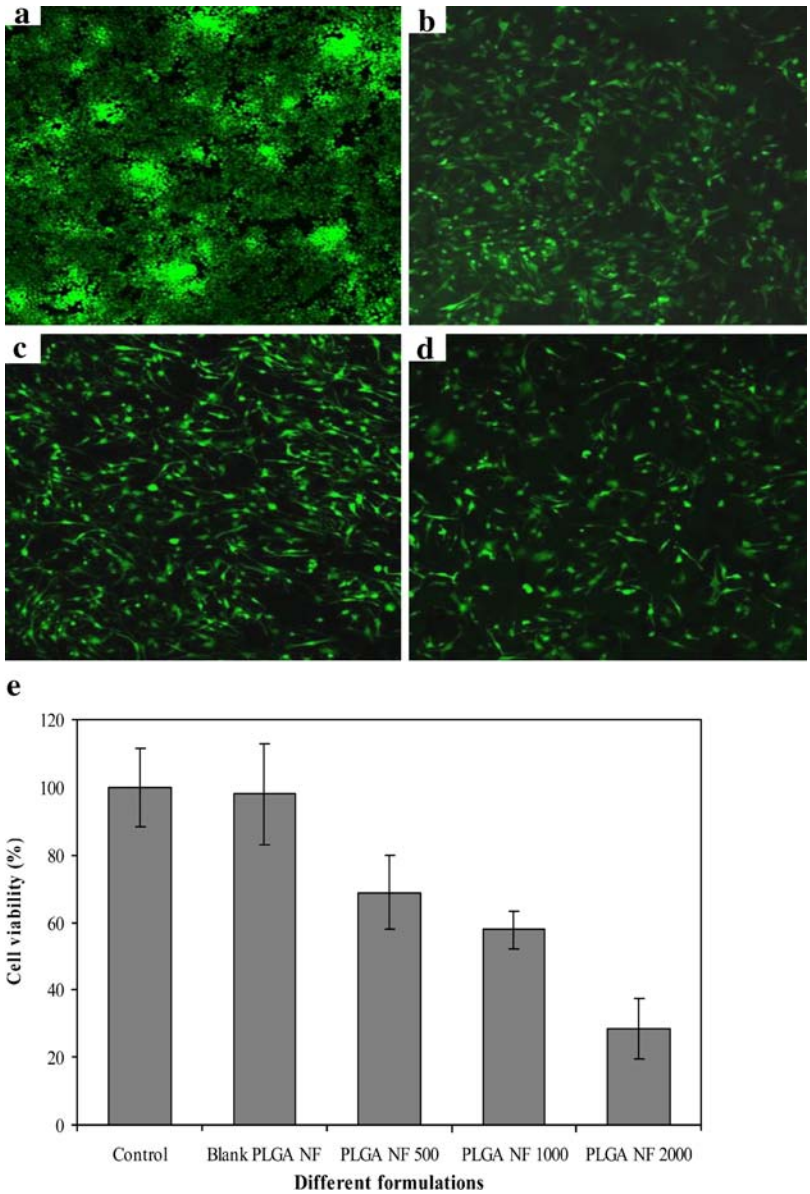

Fig. 9. (a)–(d) Laser scanning confocal microscopy images of cell morphology after 72-h incubation with different formulations. (a) Blank PLGA nanofibers; (b) s2 500 μg/ml; (c) s2 1,000 μg/ml; (d) s2 2,000 μg/ml. (10× the *green colour* indicates living cells stained with FDA.) (e) Cell viability after 72-h incubation with different formulations. PLGA NF500: 500 μg/ml; PLGA NF 1,000: 1,000 μg/ml; PLGA NF 2,000: 2,000 μg/ml.

ditions for the transport of drugs from nonswellable devices is best described by the following equation (37),

$$\frac{M_t}{M_\infty} = kt^n \tag{1}$$

where M_t is the mass of drug released at time t, M_∞ is the mass of drug released as time approaches infinity (or equivalently the total amount of drug encapsulated), k is a constant, and n is the diffusional exponent, which is an index for the transport mechanism. Paclitaxel-loaded PLGA microfibers and nanofibers *in vitro* release data were fitted with Eq. (1), thus giving

$$\frac{M_t}{M_\infty} = 0.119t^{0.37} \quad r^2 = 0.991 \tag{2}$$

For paclitaxel – loaded PLGA microfibers

$$\frac{M_t}{M_\infty} = 0.223t^{0.31} \quad r^2 = 0.998 \tag{3}$$

For paclitaxel – loaded PLGA nanofibers

For one-dimensional Fickian diffusion of drugs from mono-dispersed cylinders, n was 0.45 (37). The possible reasons for the deviation of the diffusional exponent, n, from 0.45 as shown in Fig. 8a could be explained by the following two reasons: (1) The paclitaxel-loaded PLGA micro- and nanofibers samples were composed of a fibrous mesh and the effective surface for drug release depends on the secondary structure how the individual fibres are integrated in the complex, (2) The interaction between drug and polymer matrix could be another barrier for the release due to the hydrophobic nature of paclitaxel. It is further noted that the actual release rates for both MF and NF are slower than the perfect diffusion models ($k = 0.45$). This might be primarily due to the geometrical effect as not all fibre cylinder surfaces are exposed to release medium.

In Vitro Cytotoxicity Test

In this study paclitaxel-loaded PLGA nanofibers were chosen to test the cytotoxicity on C6 glioma cells. FDA staining of living cells was first employed to study the cytotoxicity qualitatively. MTT assay was then used to quantify the cell viability after administration of different dosage forms. Figure 9a–d showed the confocal laser scanning microscope images of living cell morphology stained with FDA in green colour. This result qualitatively indicated that the cell density was much lower after administration of different concentrations of paclitaxel-loaded PLGA nanofibers, as compared to the control and blank PLGA nanofibers after 72 h. Figure 9e showed the quantitative cytotoxicity study of paclitaxel-loaded nanofibers on C6 glioma cells. In order to characterize the hydrophobic nature of paclitaxel, IC50 (around 30 μg/ml) of commercial formulation Taxol® against C6 glioma cell was tested and reported in our previous study (38). It is seen that cell viability could decrease with increasing formulation concentration and the IC50 value of paclitaxel-loaded PLGA nanofibers is around 1,200 μg/ml. According to *in vitro* release profiles, approximately 30% of drugs were released from PLGA nanofibers after 72 h. The drug loading was around 10% as shown in Table I. Therefore, the IC50 value of this formulation (36 μg/ml, calculated based on the amount of paclitaxel) is comparable to the IC50 value for commercial paclitaxel formulation Taxol®. Furthermore, for long-term treatment, higher dose of this formulation (as compared to Taxol®) can be administrated due to its sustained release property.

CONCLUSIONS

Controllable diameter from around 30 nm to 10 μm of PLGA-based micro- and nanofibers was achieved by electrospinning. A model anticancer drug—paclitaxel was encapsulated in fibers and encapsulation efficiency was more than 90%. The sustained released could last for more than 2 months. Cell viability test results suggested that IC50 value of paclitaxel-loaded PLGA nanofibers was around 36 μg/ml after 72 h incubation. Therefore, electrospun micro- and nanofibers as implants could be an alternative approach and very promising local drug delivery devices for the treatment of brain tumour.

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