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Microparticles developed by electrohydrodynamic atomization for the local delivery of anticancer drug to treat C6 glioma in vitro

Jingwei Xie[a], Jan C.M. Marijnissen[b], Chi-Hwa Wang[a,c,*]

[a]Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore
[b]Department of Chemical Technology, Delft University of Technology, Particle Technology Group, Julianalaan 136, 2628 BL, Delft, The Netherlands
[c]Molecular Engineering of Biological and Chemical Systems, Singapore-MIT Alliance, Singapore 117576, Singapore

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Abstract

This study aims to fabricate biodegradable polymeric particles by electrohydrodynamic atomization (EHDA) for applications in sustained delivery of anticancer drug—paclitaxel to treat C6 glioma in vitro. Controllable morphologies such as spheres, donut shapes and corrugated shapes with sizes from several tens of microns to hundred nanometers of particles were observed by scanning electron microscopy (SEM) and field emission electron microscope (FSEM). The differential scanning calorimetry (DSC) study indicated that paclitaxel could be either in an amorphous or disordered-crystalline phase of a molecular dispersion or a solid solution state in the polymer matrix after fabrication. The X-ray photoelectron spectroscopy (XPS) result suggested that some amount of paclitaxel could exist on the surface layer of the microparticles. The encapsulation efficiency was around 80% and more than 30 days in vitro sustained release profile could be achieved. Cell cycling results suggested that paclitaxel after encapsulation by EHDA could keep its biological function and inhibit C6 glioma cells in G2/M phase. The cytotoxicity of paclitaxel-loaded biodegradable microparticles to C6 glioma cells could be higher than Taxol® in the long-term in vitro tests evaluated by MTS assay. The drug delivery devices developed by EHDA in this study could be promising for the local drug delivery to treat malignant glioma.

Keywords: Electrohydrodynamic atomization; Biodegradable; Microparticle; Paclitaxel; Cytotoxicity

1. Introduction

Current treatments for malignant glioma sometimes remain unfavorable, and the duration of survival time of patients has made limited progress during the past years, despite a lot of advances in surgery, radiotherapy, adjuvant chemotherapy and imaging techniques [1]. The localized drug delivery device has many advantages to treat malignant glioma. It can be implanted directly at the tumor site and the released drug can bypass the blood–brain barrier and thereby increase the concentration of drugs in the brain [2,3]. As a result, both systemic exposure and side effects of chemotherapy are minimized. A few types of localized drug delivery devices including wafers, discs and microparticles have been developed for the local treatment of various tumors [4–12]. For wafers and discs, open surgery for implantation is always required for treatment of malignant glioma. In contrast, microparticles could be easily implanted by stereotaxy in discrete, precise, and functional areas of the brain without causing damage to the surrounding tissue [13]. Harper et al. reported that paclitaxel-loaded p(DAPG-EOP) microspheres are safe and potent for regional therapy of lung cancer tumor nodules in mice [9]. Nsereko et al. studied paclitaxel-containing chitin and chintin-Pluronic® F-108 microparticles and confirmed their use as biodegradable systems for localized administration in murine model of Lewis lung carcinoma [10]. Attawia et al. reported that paclitaxel-loaded PMA-CPH microspheres can be used in conjunction with irradiation for the treatment of Ewing's sarcoma to effectively release paclitaxel while maintaining its

*Corresponding author. Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore. Tel.: +65 6874 5079; fax: +65 6779 1936.
E-mail address: chewch@nus.edu.sg (C.-H. Wang).

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combined cytotoxic and radiosensitizing abilities [11]. In a separate study where 9L glioblastoma cells were introduced into the abdominal cavity of Wistar rats, Demetrick et al. found that the paclitaxel-loaded PLA microsphere formulation was more effective than conventional paclitaxel in preventing tumor seeding [12].

There are many methods to fabricate microparticles such as solvent evaporation [14], spray drying [15], spraying a polymer solution through a small orifice [16,17] and Shirasu Porous Glass (SPG) membrane emulsification technique [18], etc. However, most of these methods have some disadvantages such as low encapsulation efficiency or tedious separation procedure of particles from aqueous phase.

The electrospray technique was widely employed to fabricate inorganic nanoparticles, thin films, fibers, hollow fibers, deposition of nanoparticle clusters, micro/nanoencapsulation and production of pharmaceutical particles [19–25]. Ding et al. [26] reported an electrohydrodynamic atomization (EHDA) technique to fabricate uniform microparticles in the range of 1–15 μm. However, the yield for particles obtained at the collection station was not satisfactory and the particle morphology was not controllable. Extending from the work by Ding et al. [26], the main objective of this study was to examine the morphology and characterization of EHDA particles obtained under various operating factors such as polymer solution flow rate, polymer concentration, type of polymers, and type of organic solvents. In performing these experiments, the present study aimed to fine tune the particle size range by systematic variation of these operating factors. Delivery and cytotoxicity of paclitaxel to C6 glioma were evaluated in vitro by downstream characterization through cell culture experiments. Cell cycling analysis and in vitro cytotoxicity were compared with the case of systemic delivery where paclitaxel has considerable side effects and a short lifetime in serum [27].

2. Materials and methods

2.1. Materials

Paclitaxel samples used in the present study were kindly offered by Bristol-Myers Squibb (New Brunswick, NJ) at no cost. Taxol® was purchased from Bristol-Myers Squibb (Princeton, NJ). DCM (Cat. No. DR-0440) and acetonitrile (Cat. No. AS-1122) were purchased from Tedia Company Inc. (Fairfield, OH). Polymers such as poly(D,L-lactide-*co*-glycolic acid) (PLGA) with L:G molar ratio of 50:50 (MW = 90,000–120,000), poly(DL-lactide) (PDLA) (MW = 106,000), poly(L-lactide) (PLLA) (MW = 85,000–160,000), polycaprolactone (PCL) (MW = 65,000) and polycaprolactone (PCL) (MW = 14,000) were purchased from Sigma Aldrich (St. Louis, MO USA). Ethylene/vinyl acetate copolymer (EVAC) (Cat# 787) was purchased from SP Scientific Polymer Products Inc. (NY, USA). Phosphate buffer saline (PBS) for in vitro release study was bought from Sigma Aldrich, containing 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.4. Propidium iodide (PI, Cat. No. P1304MP) was obtained from Molecular Probes Inc. (Eugene, OR). CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay was bought from Promega Corporation (Madison, WI). All other materials and reagents used were of analytical grade.

2.2. Microparticle fabrication by electrospray

The experimental setup is shown in Fig. 1a. A well-defined potential difference is created between a nozzle and a ring, by applying a high voltage on the nozzle and a lower high voltage on the ring. Through the nozzle a solution, containing dissolved polymer and drug, is pumped at a low predetermined rate. At the nozzle tip a liquid cone is formed, with a thin jet emerging from the apex. This jet breaks up in mono-dispersed droplets [28,29]. The charged droplets need to be neutralized to make them manageable and to avoid coulomb fission, due to solvent evaporation. Neutralization is accomplished by a corona discharge system, which is created at the tip of a sharp, grounded needle, positioned opposite the high-voltage ring and needle. Fig. 1 shows both spraying in a single-jet mode (Fig. 1b2) and in a multiple-jet mode (Fig. 1b1). Going from the cone-jet mode to the multiple-jet mode is accomplished by increasing the potential difference between the nozzle and the ring. This is done here by keeping the potential of the nozzle constant and decreasing the potential of the ring.

2.3. Scanning electron microscopy (SEM)

The morphology of microparticles observed by SEM (Jeol JSM 5600LV) required an ion coating with platinum by a sputter coater (JFC-1300, Jeol, Tokyo) for 40 s in a vacuum at a current intensity of 40 mA after mounting the sample on metallic studs with double-sided conductive tape. The accelerating voltage ranged from 10 to 15 kV during scanning.

2.4. X-ray photoelectron spectroscopy (XPS)

The surface chemistry of the microspheres was determined by AXIS His X-ray photoelectron spectroscopy (Kratos Analytical Inc., NY) using the curve fitting software provided by the manufacture. For all samples, a survey spectrum was recorded over a binding energy range of 0–1100 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 (N1s 399 eV) at the surface.

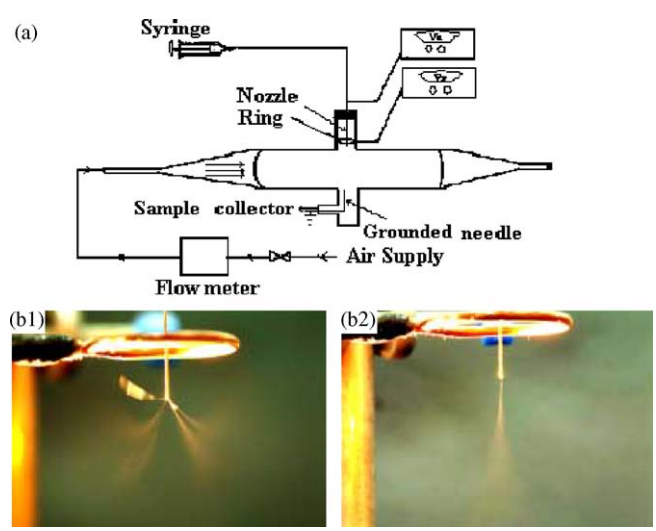

Fig. 1. (a) Diagram of electrohydrodynamic atomization experimental setup; (b1): multiple-cone spraying (nozzle voltage: 10.0 kV; ring voltage: 0 kV); (b2): single-cone spraying (nozzle voltage: 10.0 kV; ring voltage: 9.0 kV). Operating parameters are the same as that of S2 sample. The voltage at the grounded needle is taken as the reference point, 0 V.