Characterization of the Size, Shape, and Drug Encapsulation Efficiency of PLGA Microcapsules Produced via Electrojetting for Drug Delivery to Brain Tumors

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ABSTRACT— Despite significant progress in the development of new chemotherapeutic agents and drug delivery methods for brain tumors, malignant gliomas (high grade brain tumor) remains deadly with a median one-year survival time. A major unmet challenge in the treatment of malignant gliomas is the development of effective and targeted local delivery of chemotherapeutic agents at the cellular level. Here, we report the results of a systematic study of the size, shape, and drug release profiles of Poly(lactic-co glycolic) (PLGA) microcapsules produced and loaded with the anticancer agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) using an electrojetting technique. We quantify the shape and size distribution of BCNU-loaded PLGA microcapsules as a function of the polymer concentration and flow rate used during electrojetting, and measure drug release profiles for microcapsules of three different morphologies: flattened microspheres, microspheres, and microfibers. The BCNU release profiles for three microcapsule morphologies are found to be in good agreement with model predictions for drug release as a result of drug diffusion and degradation of PLGA.

I. INTRODUCTION

ANTINEOPLASTIC agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), also called Carmustine, has been widely used in treating several types of brain tumors [1]. BCNU poses an outstanding ability to cross the blood brain barrier (BBB), because of its good lipid solubility and relatively low molecular weight.[2] The traditional method of delivering BCNU is typically through intravenous perfusion [2]. The drawbacks associated with this method are the sharp initial increase in drug concentration above the therapeutic range and its relatively short (<15 min) half-life [3]. In order to increase long-term maintenance of therapeutically desirable levels, eliminate the dose-related side effects, reduce the required dosage and number of applications (leading to patient convenience and compliance), and enhance the drug’s long-term biological activity, BCNU has been incorporated into polymeric matrices and tested for efficacy against intracranial tumors [4, 5]. Biodegradable polymers release drugs by a combination of polymer degradation and drug diffusion [6, 7]. Hence, they offer great promise for sustained delivery of antineoplastic agents with desirable nearly zero-order release [8].

Poly (lactic-co-glycolic) (PLGA) has been widely used as a biocompatible polymeric material for controlled drug delivery systems based on the incorporation of drugs into polymer matrices [9]. Biocompatibility of monomers (lactic acid and glycolic acid) is the foundation for biocompatibility of PLGA. In addition, the degradation rate of PLGA can be precisely tailored by varying the ratio of lactic acid to glycolic acid [10]. Drug-loaded PLGA microcapsules can be formed by spray drying and emulsion evaporation methods [11]. However, there are some disadvantages associated with these methods, such as polydispersity, low drug loading capacity, and low drug encapsulation efficiency of microcapsules, presumably due to the multiple steps of the fabrication process [12]. These challenges directed us toward producing BCNU-loaded PLGA microcapsules using an electrojetting technique. Electrojetting is a simple, cost-effective, and versatile method for generating micro- and nano-meter sized capsules from solutions of polymers and ceramics [13, 14]. In the electrojetting technique, a conducting liquid is injected at a constant flow rate through a high-voltage electrified capillary tube. A conical meniscus is formed at the tube exit, which ultimately pinches off to produce a stream of fibers (electrospinning) or droplets (electrospraying).

II. MATERIALS AND METHODS

A. Material

Poly(D,L lactide-co-glycolide) 50:50 with an average molecular weight of 106,000 was purchased from Boehringer Ingelheim Pharma, Ingelheim, Germany. BCNU was purchased from Sigma–Aldrich. All other chemicals and reagents were purchased from Sigma–Aldrich.

B. Preparation of BCNU-loaded PLGA microcapsules

To prepare PLGA-BCNU solutions with PLGA concentrations ranging from 1 to 10 wt%, 0.15-1.65 g of PLGA was first dissolved in 10 mL of chloroform at room temperature for 10 h in order to form a homogenous solution. Subsequently, 37-83 mg of BCNU (5-25 wt% with respect to PLGA) was added to the PLGA solutions. The
mixture solutions were then electrojetted on gold substrates using an applied electric field of 100 kV/m (10 kV applied voltage and 10 cm distance between the syringe needle and the substrate). BCNU-loaded PLGA microcapsules were deposited directly on the surface of aluminum wafers (2 cm × 2 cm). All electrojetting processes were carried out at room temperature in a closed plexiglass box.

**C. Shape, size, and surface characterization**

The surface morphology of BCNU-loaded microcapsules was characterized using Scanning Electron Microscopy (FEI NanoSEM 630 FESEM). Before each characterization experiment, gold electrodes with PLGA microcapsules were left in a fume hood for 12 h to evaporate any residual organic solvent. Later, particle size was assessed by analyzing the SEM micrographs with Image J analysis software (NIH).

**D. In vitro release of BCNU-loaded microcapsules**

To prevent degradation of BCNU, microcapsules were instantaneously transferred to a phosphate buffered saline (PBS) for release experiments. Three experimental groups (flattened microspheres with drug to polymer ratio of 1:4, microspheres with drug to polymer ratio of 1:7.5, and microfibers with drug to polymer ratio of 1:20) were considered (five samples for each group). Samples were placed in a Petri dish filled with 10 mL of PBS (pH 7.4). The petri dishes were incubated at 37°C. After the required incubation time, samples were dipped in deionized water. To dissolve BCNU-microcapsules, samples were placed into glass vials containing 20 mL chloroform for 30 min. After dissolving the BCNU and microcapsules, aluminum foils were removed from the bottles, and chloroform was evaporated. PBS (2 mL) was added to residuals, and the solutions were analyzed for BCNU concentration using a UV spectrophotometer (Shimadzu, UV-1700) at a wavelength of 239 nm. The amount of released BCNU was calculated as the difference between the initial and measured mass of BCNU in the microcapsules. The released values were calculated according to a prepared standard curve.

**E. Statistical analysis**

Statistical analysis of data was performed by one-way ANOVA (Origin 8.6 SRO, Northampton, MA). Statistical significance in all cases was defined assuming a confidence level of 95% (p < 0.05). All data were expressed as mean ± standard deviation (SD).

**III. RESULTS AND DISCUSSION**

**A. Shape and size of BCNU-PLGA microcapsules**

We precisely quantified the shape and size of electrojetted BCNU-PLGA microstructures as a function of polymer concentration (1 to 10 wt% PLGA) and flow rate (0.25 to 1.0 mLh⁻¹) (Fig. 1). Flattened microspheres were produced from 1 and 2 wt% PLGA solutions. Increasing the PLGA concentration from 3 to 5 wt% formed Microspheres. Upon further increasing the PLGA concentration to 6 wt%, microspheres were electrospayed with a certain degree of concomitant nanofiber (440 ± 50 nm) formation in between and on top of the microspheres [15]. Beaded-microfibers were formed using concentration between in the range of 7 wt% to 9 wt%. Eventually, microfibers were produced as the PLGA concentration was increased to 10 wt%.

We investigated the effect of flow rate on the size and monodispersity of BCNU-loaded PLGA microcapsules. Fig. 1b illustrates the variation in the size of microcapsules for the three different flow rates 0.25, 0.50, and 1.0 mLh⁻¹. As the flow rate increased, microcapsules grew in size. For example, microspheres ranging in diameter from 5.2 ± 0.3 to 8.4 ± 1.0 μm were formed by increasing the flow rate from 0.25 to 1.0 mLh⁻¹, respectively (Fig. 1b, type 4). For a fixed flow rate, there was a statistically significant difference (p < 0.05) between the sizes of flattened microspheres in types 1 and 2, and microspheres in types 3 and 5. There was also a statistically significant difference (p < 0.05) among the size distributions of microcapsules of each type of flattened microspheres and microspheres at different flow rates, except for type 4 microspheres at flow rates of 0.25 mLh⁻¹ and 0.5 mLh⁻¹ (Fig. 1b). As shown in Fig. 1b, there was also a statistically significant difference (p < 0.05) between the size distributions of microfibers formed at flow rates of 0.25 mLh⁻¹ and 1.0 mLh⁻¹ (type 10).

We also characterized the surface morphology and monodispersity of microcapsules. The SEM image in Fig. 2a-f shows PLGA microcapsules attached on the surface of the electrode. We quantified the monodispersity of BCNU-loaded PLGA microcapsules by calculating the coefficient of variation (CV) of the size of microcapsules defined as CV = σ /< d >, where < d > and σ denote the mean and standard
deviation of the microcapsule diameter, respectively. A commonly accepted definition of monodispersity is $CV < 5\%$ [16]. As shown in Fig. 2g-i, the BCNU-loaded flattened microspheres, microspheres, and microfibers produced with a flow rate of 0.25 mL h$^{-1}$ exhibited narrow polydispersity in size ($CV < 5\%$). In general, microcapsules of type 1, 4, and 10 formed at a flow rate of 0.25 mL h$^{-1}$ exhibited narrower size distributions ($CV = 4.6\%$, $CV = 4.5\%$, and $CV = 4.8\%$, respectively, Fig. 2g-i) compared to other types of microcapsules.

B. Sustained drug release and mathematical modeling

We investigated the release profile of BCNU from microcapsules containing 0.63 mg of BCNU. There was an initial burst, followed by a long period of slow release for microspheres. As the PLGA microspheres are placed in an aqueous solution, water initially penetrates into the exposed surface of the microsphere, and the BCNU trapped within a thin surface layer is quickly released (initial burst in Fig. 3b). Subsequently, water penetrates into the bulk of the PLGA microsphere, causing polymer bond cleavage and bulk erosion. The burst release might have also been made more pronounced by: (i) an inhomogeneous distribution of BCNU in the PLGA microsphere and (ii) water uptake by the PLGA microsphere due to the hydrophobic nature of BCNU and the hydrophilic nature of PLGA. (Fig. 3c)

As shown in Fig. 3d, flattened microspheres exhibited a more pronounced initial burst and higher release rate than microspheres and microfibers. Microcapsule size and drug loading capacity strongly affect the rate of drug release. For fixed microcapsule morphology, the drug release rate increases with increasing drug loading or decreasing microcapsule size. Reducing the size of a microcapsule increases its surface area-to-volume ratio, which leads to faster drug release since the drug will have a shorter distance to diffuse to get to the surface of the microcapsule. Similarly, water penetration into smaller microcapsules will be quicker, owing to the shorter distance from the surface to the center of a microcapsule. Faster water penetration into smaller microcapsules acts to accelerate the degradation mechanism, thereby leading to enhanced drug diffusion through the microcapsule. To predict the rate of drug release from BCNU-loaded PLGA microspheres, we compared the experimental drug release profile with the corresponding predictions of mathematical models for mass transfer from microspheres (Fig. 3d). A brief description of these models follows.

Microfibers:

For diffusional release of drug from a cylindrical polymeric matrix into a surrounding water phase, the equations governing the time-dependent drug concentration $C(r, t)$ within a cylindrical matrix of radius $R$ can be written

$$\frac{\partial C}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( r D_r \frac{\partial C}{\partial r} \right)$$

(1)

$$C(r, 0) = C_i; \quad C(R, t) = 0,$$

where $r$ is the radial distance from the microfiber axis, $D_r$ is the effective diffusivity of BCNU in PLGA, and $C_i$ denotes the initial uniform drug concentration within the microfiber. The zero concentration boundary condition on the surface of the microfiber assumes a well-mixed external aqueous phase such that the drug is washed away as it diffuses to the fiber surface. The large reservoir-to-microfiber volume ratio produces negligibly small drug concentrations in the external phase (about six orders of magnitude smaller than $C_i$ at steady state). For constant effective diffusivity, the predicted cumulative drug mass released from the microfiber, $M(t)$, up until time $t$ can be shown to be

$$\frac{M(t)}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{4}{\lambda_n^2} e^{-\lambda_n^2 D_r t / R^2}$$

(3)

where $M_\infty$ denotes the initial drug loading in the microfiber, the eigenvalues $\lambda_n = \{2.41, 5.52, 8.65, 11.8, \ldots\}$ are the roots of $J_0(\lambda_n) = 0$, and $J_0$ is the Bessel function of the first kind.

Microspheres and flattened microspheres:

In the case of diffusional drug release from a spherical polymer matrix of radius $R$, the governing differential equation takes the form

$$\frac{\partial C}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 D_r \frac{\partial C}{\partial r} \right),$$

(4)

where $r$ denotes the radial distance from the center of the microcapsule. The initial and boundary conditions for this case remain the same as those in Eq. (2), with the addition of a no-flux condition at the equatorial plane for flattened microspheres. Since the latter condition is identical to the symmetry condition about the equatorial plane for a microsphere, the mathematical formulations for the microsphere and flattened microsphere are equivalent. As such, the resulting expression for the cumulative drug mass released from either microcapsule up until time $t$ is given by

$$\frac{M(t)}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{6}{n^2 \pi^2} e^{-\pi^2 n^2 D_r t / R^2},$$

(5)

Model predictions based on Eqs. (3) and (5) for the cumulative drug mass released from PLGA microcapsules
are compared to experimental measurements of drug release in Fig. 3d. The solid curves in this figure represent least-squares fits to the experimental data for different microstructures, using the effective diffusivity of BCNU in the polymer matrix as a fitting parameter. The initial drug loading was determined from the long-time plateau of the experimental release profile of flattened microspheres. For all three microcapsule morphologies, there is excellent agreement between theoretical predictions and experimental measurements, indicating that drug release from the microcapsules can be satisfactorily described as a time-dependent diffusion process. The computed effective diffusivities corresponding to the solid curves in Fig. 3d are 1.5 × 10^{-14} \text{cm}^2/\text{s} for flattened microspheres, 2.1 × 10^{-14} \text{cm}^2/\text{s} for microspheres, and 3.5 × 10^{-15} \text{cm}^2/\text{s} for microfibers, well within the range of values reported in the literature for hydrophobic anti-tumor drugs [17].

IV. CONCLUSIONS

The use of an electrojetting technique enabled us to produce tunable and monodisperse BCNU-encapsulated PLGA microspheres. Preparation of microspheres with this method has several advantages, such as: 1) it offers easy control over the shape and size of microspheres, 2) it allows production of microspheres with a narrow size distribution, and 3) it enables production of BCNU-loaded microspheres with high drug encapsulation efficiency. To our knowledge, highly efficient encapsulation of BCNU in monodisperse PLGA microspheres has not been reported. Mathematical modeling of BCNU release from PLGA microcapsules was developed to predict the effective diffusivity of BCNU within the microcapsules. Finally, this drug delivery system holds a considerable promise for precise control of anticancer agent release at the tumor sites to minimize the drug side effects. Future study will focus on the effect of BCNU release on brain tumor cells.

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