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# Paclitaxel and suramin-loaded core/shell microspheres in the treatment of brain tumors

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ABSTRACT

This work presents a modified method, namely coaxial electrohydrodynamic atomization, for the preparation of microspheres with distinct core/shell structures. This allows the encapsulation of two drugs with different characteristics in hydrophilic properties in one single step. Variation of ratios between outer flow and inner flow produces polymer microspheres with different core/shell ratios, and consequently results in variable release rates of drugs. Significant changes in release patterns were demonstrated when the distributions of the two drugs in microspheres were swapped. Moreover, cell culture experiments and animal experiments have been carried out to testify the performances of different microspheres in cytotoxicity, cellular apoptosis *in vitro* and tumor inhibition against subcutaneous U87 glioma xenograft in BALB/c nude mice. These findings present the advantages and possible application of this kind of multi-drug release system in treating brain tumors. Moreover, the release rates and characteristic sequences of multi-drugs can be tailored and tuned according to treatment necessity and applied in treating other kinds of tumors.



## 1. Introduction

Malignant gliomas such as glioblastoma multiforme being characterized by aggressive proliferation of undifferentiated cells, pervasive invasion into distant healthy brain tissue and a high penchant to recur is among the most recalcitrant tumors to be treated. In spite of access to state-of-the-art imaging, neurosurgery, radiotherapy and chemotherapy, patients have a median survival time of around 3 months and most of them die within 6 months to 2 years post-diagnosis [1,2]. This is probably suggestive of the fact that local recurrence of the tumor is the primary etiology of death which is confirmed from observed local glioma recurrence within 2 cm of the original tumor resection field [3]. Thus by inhibiting local recurrence, improvement in the patient's survival seems achievable. Chemotherapy through local intracranial drug delivery using biodegradable polymeric wafers (Gliadel®) delivering BCNU showed promise [4–7]. However, most current biodegradable delivery systems deal with the delivery of one single anti-tumor agent, which is not practicable for cancer therapy in most cases [8–14]. Anti-tumor treatment is a multistage process that is regulated by a serial of inhibition factors. As each factor exhibits a distinct time-dependent concentration profile during the inhibition process, sequential or coupled release of multiple inhibitors with specific temporal profiles is expected to be critical to the successful tumor inhibition [15–18].

Paclitaxel is a well-known mitotic inhibitor and radiosensitizing agent used in cancer chemotherapy [19]. Its mechanism of action comprises of interaction with microtubule degradation resulting in increased mitotic arrest, decrease in cellular motility, disruption in intercellular signal transmission and binding to Bcl-2 inducing apoptosis [20–22]. Despite its known potent cytotoxicity against malignant gliomas *in vitro* [23,24], paclitaxel administered systemically has failed to prolong survival of glioma patients [25–27] mainly due to its hydrophobic nature and fast clearance from the plasma [28] and poor absorption across the blood brain barrier (BBB) due to P-gP efflux effects [29]. Also, its commercial form, Taxol® formulated with a 50:50 mixture of non-ionic surfactant Cremophor EL and dehydrated ethanol is often associated with anaphylactic hypersensitivity reactions and neurotoxicity when used systemically [30]. Thus, locally delivered paclitaxel offers advantages over other drugs and development of efficient paclitaxel delivery implants would augment the efficacy of chemotherapy against glioma [14].

Suramin is a polysulphonated naphthylurea with potential antineoplastic activity. Suramin blocks the binding of various growth factors, including insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), platelet-derived growth factor

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**Table 1**
Definition of samples in cytotoxicity and apoptosis analysis.

| Samples | Blank ctrl | Blank Particles | Tax (a) | Tax (b) | SRM (a) | SRM (b) | Ctrl_A (P/O) | Ctrl_B (S/O) | A (P/S) | B (S/P) |
|---|---|---|---|---|---|---|---|---|---|---|
| | No drug | Blank particles | Free Tax | Free Tax | Free SRM | Free SRM | Core: PLLA/paclitaxel Shell: PLGA | Core: PLLA/suramin Shell: PLGA | Core: PLLA/paclitaxel Shell: PLGA/suramin | Core: PLLA/suramin Shell: PLGA/paclitaxel |

(PDGF), and tumor growth factor-beta (TGF-beta), to their receptors, thereby inhibiting endothelial cell proliferation and migration. This agent also inhibits vascular endothelial growth factor (VEGF)- and basic fibroblast growth factor (bFGF)-induced angiogenesis; retroviral reverse transcriptase; uncoupling of G-proteins from receptors; topoisomerases; cellular folate transport; and steroidogenesis [31–35].

Clinical reports suggest that paclitaxel and suramin have additive effect on treating solid tumors [15,16,18]. However, high initial concentration or/and a fast release or of suramin is likely to impose serious toxicity to surrounding normal cells. Thus, similar to the case of paclitaxel, a high initial concentration of suramin or a fast release of it is not recommended in the literature. In order to tackle these challenges, microspheres releasing multiple drugs in a controlled manner are highly demanded. Different formulations of microspheres with distinct and adjustable distributions of drugs and variable release profiles would allow for multiple drugs to be delivered according to customized requirements. This could enable the practicable development of treatment devices for cancer therapy, which would be more clinically relevant than those offered by existing delivery systems.

In our previous work, a method called coaxial electrohydrodynamic atomization was utilized for preparation of core/shell microspheres with well-defined and adjustable compartments. Variation of ratios between outer flow and inner flow produces polymer microspheres with varied core/shell ratios, and consequently results in varied release of drugs. These findings present high flexibility in designing new release systems for multi-drugs. In the current work, different microspheres will be fabricated and their performances *in vitro* and *in vivo* will be comprehensively investigated.

## 2. Experimental section

### 2.1. Materials

PLLA (Av. Mol. Wt., 85,000–160,000 Da) and PLGA copolymer with lactide/glycolide molar ratio of 50:50 (Av. Mol. Wt., 40,000–75,000 Da), anhydrous ethanol and suramin sodium salt were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Dichloromethane (DCM) and ethyl acetate (EA) were both procured from Tedia (Fairfield, OH, USA). Paclitaxel used in the present study were kindly offered by Bristol-Myers Squibb at no cost and Taxol® was purchased from Bristol-Myers Squibb (New Brunswick, NJ, USA). Commercial Spectro/Por® membrane (molecular weight cut-off: 3500) used in release studies was acquired from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

### 2.2. Microsphere fabrication

The coaxial needle (Popper and Sons, Lake Success, NY, USA) is made of 316L stainless steel. The outer capillary has an outer diameter of 3 mm and an inner diameter of 2 mm. The core capillary has a diameter of 0.72 mm. Two syringe pumps (KD Scientific, Holliston, MA, USA) deliver the polymer solutions at a specific rate into the inner and outer capillary of the coaxial needle. A voltage generator (Glassman High Voltage Inc., High Bridge, NJ, USA) supplies a high voltage to the nozzle by means of a crocodile clip. In order to stabilize the electric field around the nozzle, another high voltage is applied to the ring (5 cm in diameter) surrounding the nozzle. By increasing the nozzle voltage ($V_{nozzle}$) and ring voltage ($V_{ring}$), the emerging droplets were gradually accelerated by the potential difference until a stable Taylor cone jet can be visually observed. In order to avoid the agglomeration of microspheres, a petri dish filled with anhydrous ethanol was utilized to collect the microspheres, substituting the aluminium foil normally used.

The objective is to fabricate, by coaxial EHDA, microspheres with distinct core/shell structures. In order to ensure that the drug in the core is not released prematurely, the core must be of slower degrading material than the shell. Hence PLLA was chosen for the core and PLGA for the shell in this study. Ideally, paclitaxel and suramin are encapsulated in PLLA core and PLGA shell respectively or the reverse in radial distribution, with suramin inside the core and paclitaxel in the shell. Therefore, the release of dual-drugs can be predicted and tailored. For the former case, paclitaxel was dissolved in PLLA/DCM solution (inner phase). As suramin sodium salt can't be dissolved in PLGA/EA solution directly, suramin was dissolved in 95% ethanol first before it was loaded into the shell phase (PLGA/EA). For the latter case, paclitaxel was dissolved in PLGA/EA for shell phase. Meanwhile, suramin was dissolved in DI water and then mixed with PLLA/DCM solution by mild ultrasonic. For both cases, the CEHDA operating parameters are around 6.5 KV for the nozzle and 3.5 KV for the ring. The microspheres collected in anhydrous ethanol were centrifuged and the resultant pallets were dried in a freeze drier.

### 2.3. Microsphere characterization

The size and general morphology of fabricated microspheres were observed by scanning electron microscopy. Mean diameters and standard deviation were obtained by the software, SmileView (Version 2.0, Jeol).

### 2.4. In vitro characterization

#### 2.4.1. In vitro release of drugs from microspheres

For *in vitro* release testing, 20.0 mg of paclitaxel and suramin-loaded microspheres was loaded in a Spectro/Por membrane bag and subsequently stored the bag in a NALGENE® Oak Ridge centrifuge tube (Thermo Fisher Scientific, Rochester, NY, USA) with 20.0 ml of PBS (pH 7.4) containing 1.0% of Tween 80. The whole system was then placed in an orbital shaker bath (GFL® 1092, Burgwedel, Germany) maintained at 37 °C and 120 rpm. At given time intervals, 1.0 ml of the incubated medium was withdrawn and replaced by 1.0 ml of fresh medium. Paclitaxel in the release samples 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 fully evaporated. The concentrations of paclitaxel and suramin were both determined by HPLC, in which a C-18 Column was used and the mobile phase was delivered at a rate of 1 ml/min 100 μl of sample was injected by an auto-sampler and the column effluent was detected using an ultra violet (UV) detector at 227 nm and 313 nm for paclitaxel and suramin, respectively.

#### 2.4.2. Cell culture and maintenance

The cell line used, U87 MG-luc2, was originated from human U87 glioblastoma multiforme cells (ATCC® Number: HTB-14) [36], which was stably transfected with firefly luciferase gene (luc2). U87 MG-luc2 was purchased from Caliper Life Sciences, Hopkinton, MA. The cells were grown in Minimum Essential Medium (Invitrogen) with 10% fetal bovine serum (Gibco, Invitrogen) and 1% penicillin–streptomycin (Gibco, Invitrogen) in humidified atmosphere containing 5% $CO_2$ at 37 °C. After

**Table 2**
Definition of samples in animal experiments.

| Sampless | Blank | Placebo | Ctrl_A (P/O) | Ctrl_B (S/O) | A (P/S) | B (S/P) |
|---|---|---|---|---|---|---|
| | C/S[a] = 1.2 ml/h/2.0 ml/h | | | | | |
| | No implantation | Core: PLLA Shell: PLGA | Core: PLLA/paclitaxel Shell: PLGA | Core: PLLA/suramin Shell: PLGA | Core: PLLA/paclitaxel Shell: PLGA/suramin | Core: PLLA/suramin Shell: PLGA/paclitaxel |

[a] C/S refers to volume flow rate ratio between core and shell.