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In-vitro evaluation of MPA-loaded electrospun coaxial fiber membranes for local treatment of glioblastoma tumor cells

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ABSTRACT

Core-sheath fibers containing a drug for brain tumor are reported. Mycophenolic acid (MPA), a FDA-approved immunosuppressant, has been demonstrated to inhibit several types of tumor cells growth. However, the effective serum MPA concentration for anti-tumor declines quickly *in-vivo* due to degradation in the liver, which hampers the development of MPA-based anti-tumor therapy. To overcome this issue, we have formed MPA-containing electrospun fiber membranes as local drug delivery vehicle and characterized MPA release profiles based on fiber composition and geometry. Coaxial fibers with poly(ε-caprolactone) (PCL)/MPA core and PCL sheath provided a more sustained release than homogenous fibers. In particular, thicker PCL sheath with 1:10 ratio of sheath thickness to fiber diameter provides gradual release in an initial period and higher MPA release after refreshing of media. The host polymer for MPA has a significant effect on the MPA release, with PCL/MPA single fiber providing more sustained release than coaxial fibers with polyvinylpyrrolidone (PVP)/MPA core and PCL sheath. *In-vitro* glioblastoma multiforme (GBM) tumor cell culture results show strong cell suppression effect by MPA-containing fiber membranes, with coaxial fiber membranes inhibiting GBM cell growth 3-5 × more than the single fiber membranes. This indicates that MPA-containing electrospun membranes have a promising potential for local treatment of GBM.

1. Introduction

Among many aspects of nanotechnology, the development of one-dimensional (with very high ratio of length to diameter) nanofibers has produced one of the most attractive nanostructures because it provides exceptional properties and remarkable adaptability. The versatility of the electrospinning technique to produce continuous nanofibers, with diameters ranging from tens of nanometers to microns and many meters in length, has been well established during the past decade [1–3]. Electrospun nanofibers form a highly porous membrane that contains a non-woven nanofiber network. The physical and chemical properties of nanofiber can be manipulated by controlling polymer concentration, solvent selections, etc [4–7].

The versatility of the electrospinning technique is further extended by producing core-sheath fibers in a single step, using coaxial electrospinning [8–12]. In addition to the basic advantages of fiber electrospinning (such as controllability of fiber morphologies and compositions, extremely high surface area and porous structure) coaxial electrospinning enables: (a) combination of two different properties from each layer into one fiber; (b) encapsulation of multiple functional molecules into the specific layer; (c) control of their release rates by designing fiber structure and compositions. Manipulating various parameters, such as material composition, polymer concentration, flow rate ratio, enables the control of the drug release rate from a short-term (few hours) to a long-term (months) time period. Therefore, coaxial electrospinning is very attractive to develop the drug delivery system.

The first use of coaxial electrospinning for the sustained release of encapsulated bioactive agents (e.g. BSA) was reported by Jiang et al., in 2006 [13]. Since then, many related uses of coaxial fiber electrospinning in drug delivery have been reported, such as tissue engineering [14,15], gene therapy [16,17], wound dressing [18,19]. In the last few years, coaxial fiber electrospinning for local chemotherapy has emerged as an attractive potential treatment

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option because it can decrease cytotoxicity during systemic chemotherapy, while also providing long-term efficacy of encapsulated anti-cancer drugs. For example, Yan et al. (2014) demonstrated the use of biocompatible coaxial fibers (PVP core – chitosan sheath) loaded with doxorubicin (DOX) anti-cancer drug against ovary cancer cells *in vitro* [20]. Also, Yang et al. (2015) developed coaxial fibers (PVA/DOX/micelles core – crosslinked gelatin sheath) as a local chemotherapy vehicle [21]. However, these reports used highly toxic anti-cancer drugs, which cause a severe side effect on normal cells near the implant site. Here, we report preliminary results on the local drug delivery strategy using coaxial nanofiber membranes incorporating an FDA-approved non-toxic drug against the glioblastoma multiforme (GBM), the most malignant brain cancer cell.

Mycophenolic acid (MPA - $C_{17}H_{20}O_6$, ~320 g/mol; Water solubility: 35.5 mg/L) is an immunosuppressant drug used to prevent rejection of organ transplants and has been used for more than two decades [22–24]. The molecular target of MPA is IMPDH (inosine monophosphate dehydrogenase), which is one of the key enzymes for GTP biosynthesis. In addition, anti-proliferative effects of MPA have been reported in cell lines obtained from a number of different malignancies. In a recent review, it has been noted, based on published work, that various tumors elevated IMPDH levels for their malignant growth and invasion, indicating that MPA has a great potential for many tumor treatments [25]. For instance, MPA treatment suppresses cell proliferation of leukemia, lymphoma, pancreatic cancer, non-small-cell lung adenocarcinoma and colon cancer cell lines [25–30]. However, bioavailability of MPA is relatively poor *in vivo* due to the high clearance by liver, hampering clinical application for cancers. In the human body, serum MPA levels that could suppress tumor growth are quickly decreased within 1 h [31,32] and mice adenocarcinoma model showed that orally taken mycophenolate mofetil (MMF), a pro-drug form of MPA, has poor tumor suppressive activity [33]. Thus, there is a critical need to develop local MPA treatment modality, which would maintain local MPA concentration for long-term period to suppress tumor growth.

Glioblastoma multiforme (GBM) was selected in this study because GBM is the most malignant primary brain tumor and its malignancy is mostly caused by local recurrence greater than 90% [34,35]. Although much effort has been devoted to developing pharmaceutical inhibitors for GBM treatment, only limited success has been achieved [36–38]. Since the GBM recurrence appears mostly within 2 cm to the original lesion [39,40], local treatment is one of the most effective ways to reduce the recurrence rate. Currently, local treatment for GBM has been explored in the clinic using Gliadel® BCNU (bis-chloroethyl-nitrosourea) wafer [41,42]. However, there are limitations and usage issues, such as an intrusive form factor of stiff BCNU wafers (~2 cm in diameter) and a relatively short effective period. BCNU wafer therapy releases most of the drug within first 5–7 days [43]. Ranganath et *al.* demonstrated that paclitaxel-loaded single (i.e. homogenous) fibers is highly effective for post-surgical chemotherapy for malignant glioma [44]. However, they did not explore the potential benefits of coaxial electrospun membranes for local GBM treatment.

The approach described here uses coaxially electrospun fiber membranes for MPA drug delivery to provide controlled and sustained MPA release for long term periods, while in close contact with the lesion area due to the physical flexibility. In this report, MPA is incorporated into homogenous ("single") and co-axial (core-sheath) fiber polymer membranes. The MPA release kinetics are investigated and the ability to suppress *in vitro* tumor cell growth is demonstrated using the most malignant GBM tumor cells. The basic approach is illustrated in Fig. 1, where MPA released from electrospun fiber membranes can inhibit the GBM cell growth. By adjusting the concentration of polymer in solution and its flow rate during electrospinning, one can control the core diameter and sheath wall thickness, which impacts the release profile of encapsulated core material.

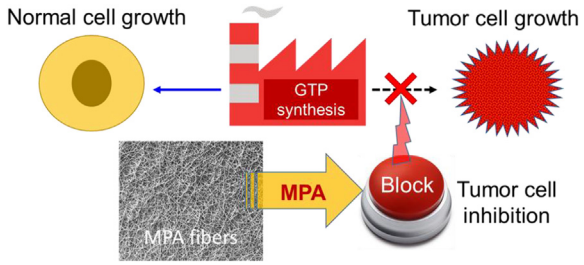

Fig. 1. Basic concept diagram of the effect of MPA on brain tumor cell.

2. Materials and methods

2.1. Materials

MPA was purchased from MB Biomedicals (Solon, OH). Two different polyvinylpyrrolidone polymers (Mw = 360 kDa and Mw = 40 kDa, denoted as PVP360 and PVP40, respectively) and poly(ε-caprolactone) (PCL, Mn = 80 kDa) were obtained from Sigma-Aldrich (St. Louis, MO). Dulbecco's Modified Eagle's Medium (DMEM) buffer, dimethylformamide (DMF, 99.9% purity), 2,2,2,-trifluoroethanol (TFE, 99.8% purity), trifluoroacetic acid (TFA), and dichloromethane (DCM) solvents were obtained from Fisher Scientific (Pittsburgh, PA). A widely used [45] GBM cell line U87MG was utilized because it is well characterized [46]. All materials were used as received without any further modification.

2.2. Methods

2.2.1. Sample preparation

Two different polymer hosts, water-soluble PVP and water-insoluble PCL, have been utilized to evaluate the effect of host polymer on the MPA release kinetics. For single electrospinning, PVP with two different molecular weights were utilized in order to obtain the optimum viscosity of PVP solution for electrospinning action at the total of 10 wt.% concentration, which enables the use of the same weight ratio (50:1) between the host polymer and MPA. To prepare solutions, both 10 wt.% of polymer (either PVP or PCL) and 0.2 wt.% of MPA drug were dissolved in the mixture of TFE and DCM. TFE was used to improve the electrospinnability by lowering the vapor pressure of the solution, while DCM was used to dissolve MPA, which is not soluble in TFE. These MPA/polymer solutions were also used for coaxial electrospinning as the core component. The sheath solution was separately prepared by dissolving PCL-only in the same solvent mixture. The list of solutions and compositions used for various fiber samples is contained in Table 1.

For coaxial electrospinning shown in Supplementary Fig. S1, both core and sheath solutions were fed at certain flow rates using syringe pumps. The total amount of dispensed solution was controlled in order to incorporate a given amount of MPA and host polymer into the fibers. The same amount of MPA was dispensed for all electrospinning cases. The electrospinning voltage level was adjusted to stabilize the Taylor cone and liquid jet actions. The distance between the nozzle and metal plate was adjusted to allow for full solvent evaporation. Overall electrospinning parameters are listed in Supplementary Table S1.