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

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A B S T R A C T

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 electrospun fibers, 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.

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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...
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.

Mycofenolic acid (MPA - C11H12O6, -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 mycofenolate 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 Gladel® BCNU (bis-chloroethyl-nitrosourea) wafer [41,42]. However, there are limitations and usage issues, such as an intruive 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.

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.
2.2. Drug release characteristics

The MPA optical absorption peak at the wavelength of 303 nm was used to characterize its release kinetics in solution (Perkin-Elmer Lambda 900 UV-vis spectrometer). The prepared membranes identified in Table 1 were immersed into 50 mL of DI water. Then, 1 mL of solution was taken at specific time intervals to measure the optical absorption of the solution. Background correction of the absorption spectrum was made with DI water. At each optical measurement, the maximum absorption intensity at 303 nm was used to quantify the released MPA amount. All measurements were performed at room temperature and relative humidity of ~40%. MPA encapsulation efficiencies of single and coaxial electrospun fibers were ~94% and ~95%, respectively, as shown in Supplementary Fig. S2.

2.2.3. Tumor cell culture

Glioblastoma multiforme cells (U-87 MG cell) were cultured with 10% FBS (Fetal bovine serum) supplemented DMEM (Dulbecco’s Modified Eagle Medium) in 24-well plates at ~25,000 per well. On the following day, MPA-containing electrospun fiber membranes (10 mm × 10 mm) were added into the wells of the plate where U87MG cells were seeded. Because the original membranes were cut into four pieces, membranes used for the cell culture were a quarter of the whole membrane weight shown in Table 1. However, the incorporated MPA amount was ~0.14 mg for all membranes. As a control, electrospun fiber membranes without MPA were also added into one well. All plates were placed in the incubator at 37 °C in an atmosphere containing 5% CO2 for 5 days. To assess cell growth, crystal violet assay was performed [47]. Briefly, cells were fixed with a 4% paraformaldehyde solution for 10 min [48], and stained with 0.4% of crystal violet dye solution for 10 min. After washing with water, cells were dissolved in 1% sodium dodecyl sulfate and the absorbance (590 nm) was measured. To observe the core-sheath structure through the fiber. In TEM, electrons are transmitted through the sample and resulting interaction forms a TEM image. Therefore, when different core and sheath materials were used, the core-sheath structure can be observed. TEM images of coaxial fibers shown in Fig. 2g and h indicate that a

2.3. Results

Single and coaxial fiber membranes incorporating MPA have been successfully produced. To investigate the effect of fiber structure and materials, single and coaxial fiber membranes with different flow rate ratios between core and sheath solutions have been prepared, as shown in Table 1. The same amount of MPA was incorporated in all membranes as demonstrated in Supplementary Fig. S2.

Fig. 2 shows typical fiber morphologies for these membranes. Fiber diameters for single fibers are slightly larger (~2.4 µm) than coaxial fibers (~1.7 µm), but this dimensional difference is not enough to explain their difference in the release rate of incorporated MPA. Fig. 2c and d shows coaxial fibers with PCL/MPA core and PCL sheath in the flow ratio of 1:1 and 1:2, respectively. Although the flow rate ratios are different, the membranes display similar fiber diameters of ~1.62 ± 0.15 and 1.69 ± 0.14 µm, respectively. Similarly, membranes consisting of fibers with PVP/MPA core and PCL sheath, shown in Fig. 2e and f, display fiber diameters which are very close at ~1.8 µm. For TEM observation, multiple fibers were collected on TEM grid during electrospinning and then measured using FEI CM20 TEM at 200 kV acceleration voltage to observe the core-sheath structure through the fiber. In TEM, electrons are transmitted through the sample and resulting interaction forms a TEM image. Therefore, when different core and sheath materials were used, the core-sheath structure can be observed. TEM images of coaxial fibers shown in Fig. 2g and h indicate that a
thicker PCL sheath layer (~94 nm vs. 56 nm) was obtained at higher sheath flow rate. The ratio of sheath thickness to fiber diameter is ~1:16.5 for the flow rate ratio of 1:1 (core: sheath) and ~1:10.5 for the flow rate ratio of 1:2.

MPA release profiles for various fiber membranes have been characterized using UV-vis spectroscopy. MPA has an optical absorption peak at the wavelength of ~303 nm, while the absorption peak for PVP is at 260 nm. Fig. 3 contains MPA-related absorption data vs. time for several membrane types.

Fig. 3a and b clearly show that the MPA release characteristics are dependent on the fiber structure, material and sheath thickness in coaxial fibers. For single-type fiber, water-soluble PVP membranes provide maximum MPA release instantly because most of the membrane is dissolved within ~1 min. Coaxial fibers with PVP/MPA core display slower release than single PVP/MPA fibers and also generate some MPA release upon refreshing the media. However, their release rate is still relatively fast due to the high solubility of PVP in water. Using water-insoluble PCL as the polymer host in single fibers results in slightly slower release than coaxial fibers with PVP/MPA core. Coaxial fibers with PCL core have a further reduction in release rate by incorporating a conformal PCL sheath layer. The sheath layer thickness plays an important role in controlling the MPA release rate, with a thicker sheath layer yielding a slower release. In the 2nd release after refreshing media, all coaxial fiber membranes show significant additional MPA release, while single PCL fibers show the lowest level of MPA released. Coaxial fiber membranes with PVP core also showed lower MPA release than coaxial fiber membranes with PCL core after refreshing media because it released most of MPA in the initial stage as recognized in Fig. 3b. For the 3rd release, only coaxial fibers with PCL/MPA core provide additional MPA release. Interestingly, the membrane with a thicker fiber sheath layer exhibits stronger MPA release than that with a thinner sheath layer. This is probably because the remaining MPA in the coaxial sample with thinner sheath layer is nearly exhausted. Another interesting aspect is that the MPA release rate is increased after the 1st refreshing of the media solution, as shown in the accumulated release profile plotted in Fig. 3b. It is also obvious that the MPA release is approaching a linear profile as the sheath thickness is increased. Based on the obtained MPA release profiles, coaxial fibers with PCL/MPA core and thicker PCL sheath provide continuing delivery of MPA drug over the longest period.

The effect of released MPA on GBM tumor cells using in-vitro cell culture was also investigated. The results are summarized in Fig. 4. The photographs in Fig. 4a qualitatively but clearly show the effect of MPA on GBM cells in vitro. With no MPA, the stain color is obvious, indicating that abundant GBM cells are present. The MPA-incorporated fiber membranes appear colorless, indicating that very few GBM cells are present. More accurate cell counts reveal differences in efficacy between membranes with different fiber structures. Fig. 4b shows the quantitative analysis of cell cultures for a period of up to two weeks using a single set of membranes, each with a different fiber structure. With the exception of the control samples, all other membranes have equal amount of MPA drug (~0.14 mg) incorporated within their fibers. In the 1st run of tumor cell culture, all MPA-containing membranes show strong growth inhibition, while the control samples result in high cell growth rate. In the 2nd run, the same fiber membranes were used with fresh tumor cells and culture media. Coaxial fiber membranes clearly provided superior inhibition compared to the single fiber membrane, because the single fibers released most of their MPA during the 1st run. Furthermore, the coaxial fiber membranes with thicker sheath provide better inhibition than those with thinner sheath, most likely because the thinner sheath allowed for more MPA to be released during the 1st run. These results correlate well with the MPA release characteristics (optical spectroscopy measurements) from each fiber membrane.

4. Discussion and conclusions

In the present study, MPA-containing electrospun fiber membranes have been successfully fabricated using single and coaxial electrospinning and have demonstrated excellent potential as a local therapy vehicle for aggressive GBM-involved brain cancer treatments. MPA-encapsulated coaxial fibers show a more sustained release of MPA inhibiting GBM growth for longer period of time, compared to MPA-incorporated single fibers. The released MPA from electrospun membranes provides excellent inhibition performance in in-vitro GBM cell growth, indicating that the incorporated MPA was not degraded during electrospinning process.

Up to now, water lavage-mediated osmotic lysis of tumor cells

![Fig. 3. MPA release kinetics from membranes consisting of fibers of various types (single, coaxial) and core/sheath materials: (a) MPA release in the initial media; (b) integrated MPA release profile with two times refreshing media. Note: single fibers — PVP or PCL; coaxial fibers — core: PVP or PCL; sheath — PCL.](image-url)
has been used to suppress spilled tumors growth, which occurs once in a while during surgery [49]. Given that MPA has been demonstrated to inhibit other types of tumor, the local MPA release could be applicable for preventing local recurrence acceleration and metastasis of several types of solid tumors. Significant increase of the long term release to periods of several weeks are needed (for example oral dosing schedule using temozolomide [50] is 49 days). The anti-GBM effect of MPA fibers was evaluated in three independent experiments and the data shown in the graph is representative of the results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jddst.2017.05.017.

References


Fig. 4. GBM cell cultures exposed to MPA-containing fiber membranes and control samples: (a) photos of cultures after 1 week; (b) relative growth rate of tumor cell cultures exposed to MPA-loaded fiber membranes—homogenous (“single”) PCL/MPA fibers, coaxial PCL/MPA core and PCL sheath (of two thicknesses). The cultures were treated with crystal violet assay and the color intensity was related to cell concentration. The anti-GBM effect of MPA fibers was evaluated in three independent experiments and the data shown in the graph is representative of the results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)