Local intracranial drug delivery using biodegradable PLGA-paclitaxel micro/nano-fiber implants to treat malignant brain tumors

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INTRODUCTION

Malignant brain tumors (gliomas) characterized by aggressive proliferation, inexorable local recurrence and invasive nature are among the most recalcitrant cancers to treat in the human body. This necessitates the urgent need for the development of implantable, biocompatible and biodegradable delivery devices carrying cytotoxic and radiosensitizing drugs for post-surgical synergetic chemo-radiotherapy against malignant gliomas. Most of the polymeric implants delivering paclitaxel (a cytotoxic and radiosensitizing drug) developed are either micro/nano-particles, compressed micro/nano-particle discs or wafers. However, due to very high interstitial pressure in the brain/tumor, intratumoral injection of drug loaded microparticles will always run the risk of being expelled out of the target site thus negating the advantages of local delivery [1]. The other disadvantage of using microparticles is the high initial burst due to the presence of the drug on the surface which might lead to undesired neurotoxicity. On the other hand, drug loaded compressed discs and wafers have low surface area to volume ratio available for polymer degradation and drug diffusion and hence would result in low drug release rates and undesired secondary burst [2].

In an effort to improve local therapy against glioma in terms of avoiding high initial burst and providing drug release sustainability and implantability, we have developed novel poly (D,L-lactide-co-glycolide) (PLGA) micro/nano-fiber implants bearing paclitaxel by Electrohydrodynamic Atomization process (EHDA). To demonstrate proof of concept, PLGA-paclitaxel fiber discs and sheets were fabricated and characterized. The performance of the formulations in sustaining drug release and cytotoxicity were also evaluated *in vitro* through apoptosis study on C6 glioma cells. In addition, the formulations were tested against subcutaneous C6 glioma tumors in BALB/c nude mice. These studies allowed a comparison between fiber discs and sheets in terms of implantability, compactness and drug release profiles which showed that the discs were better suited. Subsequently, to better understand the contributions of the co-polymer and the fiber diameter towards the drug release, six fiber disc dosage forms with varying co-polymer and fiber diameter were fabricated and characterized in terms of in vitro release profiles.. Also, the best dosage forms for further in vivo studies were selected which exhibited small initial burst, relatively zero-order release rate and sustainability over extended periods.

EXPERIMENTAL METHODS

PLGA was dissolved in a mixture of dichloromethane (DCM) and dimethylformamide (DMF) at 30% (w/v) concentration. 9.1% (w/w) paclitaxel was then dissolved in the solution and the drug-polymer solution was pumped at a predetermined rate using a syringe pump, forming a bead of solution at the tip of syringe. A high voltage difference (12-20 kV) was applied between the nozzle (27 G needle with diameter of 0.34 mm), a negative potential and a grounded collection target. As the jet broke up into fibers from the Taylor Cone, DCM was evaporated giving rise to relatively dry fibers which were subsequently spun on the aluminum foil wrapped rotating shaft until multilayered fiber mat was obtained.

Paclitaxel-PLGA fiber discs were obtained by punching the fiber mats into 3 x 1 mm discs. The mats were cut into 5 x 5 mm sheets to obtain paclitaxel-PLGA fiber sheets. PLGA 85:15 was used to fabricate microfiber disc (MFD) and sheet (MFS), whereas PLGA 50:50 was used for submicrofiber disc (SFD) and sheet (SFS). The dosage forms were freeze dried for approximately one week to remove residual DCM. The discs were sterilized using Co-60 gamma irradiation at a dose of 15 kGy before using for in vitro and in vivo studies. To compare the effect of co-polymer and fiber diameter on drug release profile, PLGA 85:15 co-polymer was used to fabricate microfiber (E1), sub-microfiber (E2) and nano-fiber (E3) discs with fiber diameters of about 3.1 µm, 930 nm and 100 nm respectively. Similarly, PLGA 50:50 was used to fabricate microfiber (F1), sub-microfiber (F2) and nano-fiber (F3) with the same diameters as above.

RESULTS AND DISCUSSION

In vitro Characterization

Fiber Diameter, Distribution, Morphology and Paclitaxel Encapsulation Efficiency

In the proof of concept study, the reason for choosing two different PLGA co-polymers was that PLGA 50:50 having higher hydrophilic glycolic acid (G) content is known to degrade at a faster rate than PLGA 85:15 owing to faster hydrolysis. This difference in bulk degradation rates was utilized to develop faster and slower releasing formulations. Additionally, by varying the fiber diameter for the two formulations the difference in surface area to volume ratio was availed to aid faster or slower release. SEM images in Fig.1 suggest that electrospun fibers were formed with good monodispersity in fiber diameters which is confirmed in Table 1.

Table 1. Electrospinning parameters and characterization of Paclitaxel-PLGA microfibers

Samples	DCM/DMF Ratio % (v/v)	Flow rate (ml/h)	Voltage difference between nozzle & ground (kV)	Distance from needle tip to shaft (cm)	Fiber mean diameter	Paclitaxel encapsulation efficiency (%)	Paclitaxel loading (%) w/w
PLGA 85:15 Microfiber (MF)	80/20	6.00	12 -16	15	3.5 ± 0.32 µm	98 ± 4.9	8.92 ± 0.49
PLGA 50:50 Submicrofiber (SF)	70/30	0.30	16 - 18	15	930 ± 35 nm	94 ± 0.57	8.60 ± 0.06

Fig. 1 also confirmed that the surface of the fibers were smooth with high encapsulation efficiency of paclitaxel. Such a non-woven fiber was hypothesised to provide higher surface area to volume ratio for drug release when compared to conventional drug delivery discs/wafers made of either compressed drug-polymer mixture or compressed drug-polymer microparticles.

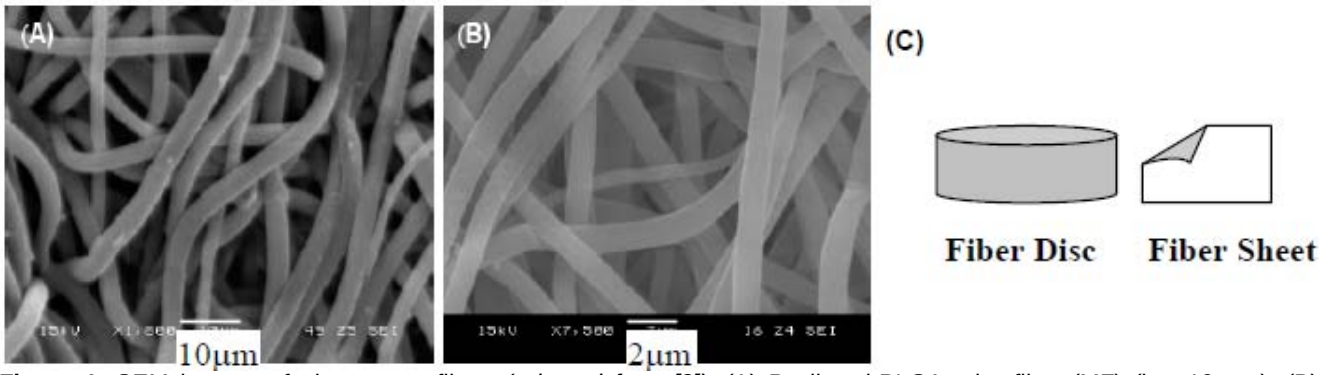

Figure 1: SEM images of electrospun fibers (a*dopted from [3]).* (A) Paclitaxel-PLGA microfiber (MF) (bar 10 µm); (B) Paclitaxel-PLGA submicrofiber (SF) (bar 2 µm). (C) Depiction of fiber disc and fiber sheet.

Later, from the two formulations (MF and SF), four dosage forms (MFD, SFD, MFS and SFS) were prepared as described earlier. A comparative study was performed on the polymer degradation, in vitro drug release, apoptotic activity and in vivo subcutaneous tumor inhibition from discs and sheets.

In vitro Release of Paclitaxel from Fiber Discs/Sheets

This study was aimed at quantifying the % cumulative release of paclitaxel from the four dosage forms under simulated physiological conditions over an extended period of time. The *in vitro* release profiles shown in Fig.2 suggest that paclitaxel release was sustained for more than 80 days for all the dosage forms. While the fiber discs showed nearly zero order release kinetics with slight initial burst (6% for MFD and SFD after 9 days), the fiber sheets (MFS and SFS) exhibited a relatively high initial burst (16% for SFS and 10% for MFS after 9 days) followed by a zero-order release. For MFD, after day 7 the release rate was almost linear and after day 80, 26% of total paclitaxel was released. For SFD, till day 31 a slow release rate was observed followed by a slight increase in release rate up to day 80. After day 80, around 35% of total paclitaxel was released from SFD. SFD and MFD also exhibit two slightly different release rates. We can notice that for SFD, the release profile from day 0 till day 35 is strikingly similar to the release profile from day 30 to day 70. Whereas for MFD, after day 64 a slight burst is seen which was similar to its initial burst.

The results from the release profile confirm the fact that SF has a higher release rate than MF for the most obvious reason of faster bulk degradation rate due to higher glycolic acid content in PLGA 50:50 compared to PLGA 85:15. This correlates well with the GPC results (not shown). MFD and SFD in our study demonstrate low initial burst probably due to the disc geometry and multilayered structure of fibers, where as

MFS and SFS due to less compact structure exhibit a relatively higher initial burst. Furthermore, SFD and MFD were made by multilayered fiber sheets, thus not all the sheets were equally exposed to release buffer. This multilayered structure made them exhibit two slightly different release rates. The reason for this was as the old layer degraded and exposed the new layer; a slight burst was seen followed by a constant release which went on till most of the polymer had degraded. Even though the discs exhibited two different release rates, they were not much different in magnitude in comparison to the biphasic release seen in the fiber sheets.

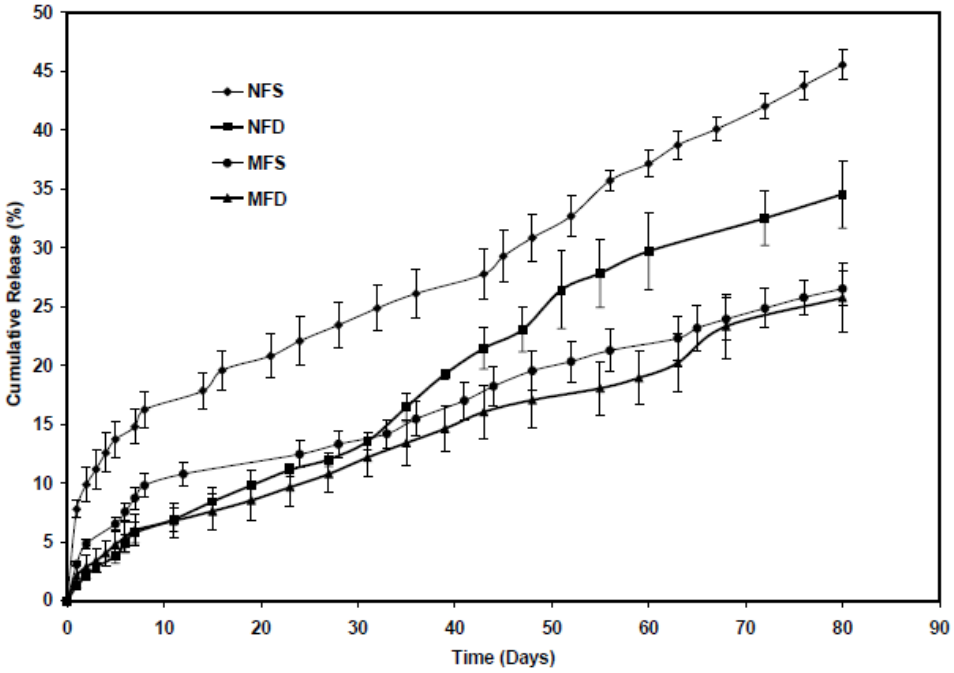

Figure 2: In vitro paclitaxel release from different dosage forms (a*dopted from [3]).* (◆) SFS; (■) SFD; (●) MFS; (▲) MFD. Each data point represents the average of triplicate samples and error bars represent standard deviation.

Other paclitaxel loaded polymeric discs/wafers like PLGA spray dried microparticle discs [2], MPEG 2000-DSPE liquid crystalline cubic phases [4], polilactofate microsphere discs [5] exhibit significantly lower release rates probably because of lower surface area to volume ratio available for polymer degradation and drug diffusion. On the other hand, other dosage forms like PLGA micro/nano-fiber films [6], PLGA microparticles by EHDA and spray drying [1], polilactofate microspheres [5], PCPP-SA microsphere discs [7] and docetaxel loaded PCPP-SA microsphere discs [8] exhibit very high initial burst in comparison to fiber discs and sheets. The large burst in some cases was due to fast degrading polymer like PCPP-SA [8] or due to surface bound drug release [1, 5]. The large burst has been reported to cause sporadic toxicity among experimental animals [7]. In comparison, the fiber discs and sheets have the advantages of low initial burst, near constant drug release rate and longer drug release sustainability which might be crucial for treating recurrent glioma. This provides the flexibility in selecting dosage forms with different drug release rates according to patient specific glioma chemotherapy (based on residual tumor post-surgery).

In vitro Cellular Apoptosis

This study was designed to demonstrate the long term paclitaxel release sustainability at a cellular level. Paclitaxel induced apoptosis was quantified for C6 glioma cells treated with MFD, SFD, MFS and SFS and compared with blank and Taxol® controls for sustained apoptotic activity. Fig.3 shows quantitative results of extent of apoptosis (caspase-3 activity) after days 4, 8 and 12. Fig.3A and 3B show low level of apoptosis in C6 glioma cells treated with blank discs/sheets, where as large apoptotic activity was observed on day 4 for Taxol® groups. For the fiber treated groups, apoptotic activity slightly higher than that of blank discs and considerably lower than that of Taxol® controls was observed. Once Taxol® was washed off and fresh media was replaced on day 3, the cells started recovering which is reflected in increased cell density (data not shown) and decreased apoptotic activity after days 8 and 12 as seen in Fig.3A and 3B. In contrast, for the fiber treated groups the cell density continued to decrease (data not shown) and apoptotic activity continued to increase after days 8 and 12. Also, SFS treated group showed the highest apoptotic activity after 12 days, whereas MFD showed the least among the fiber treated groups.

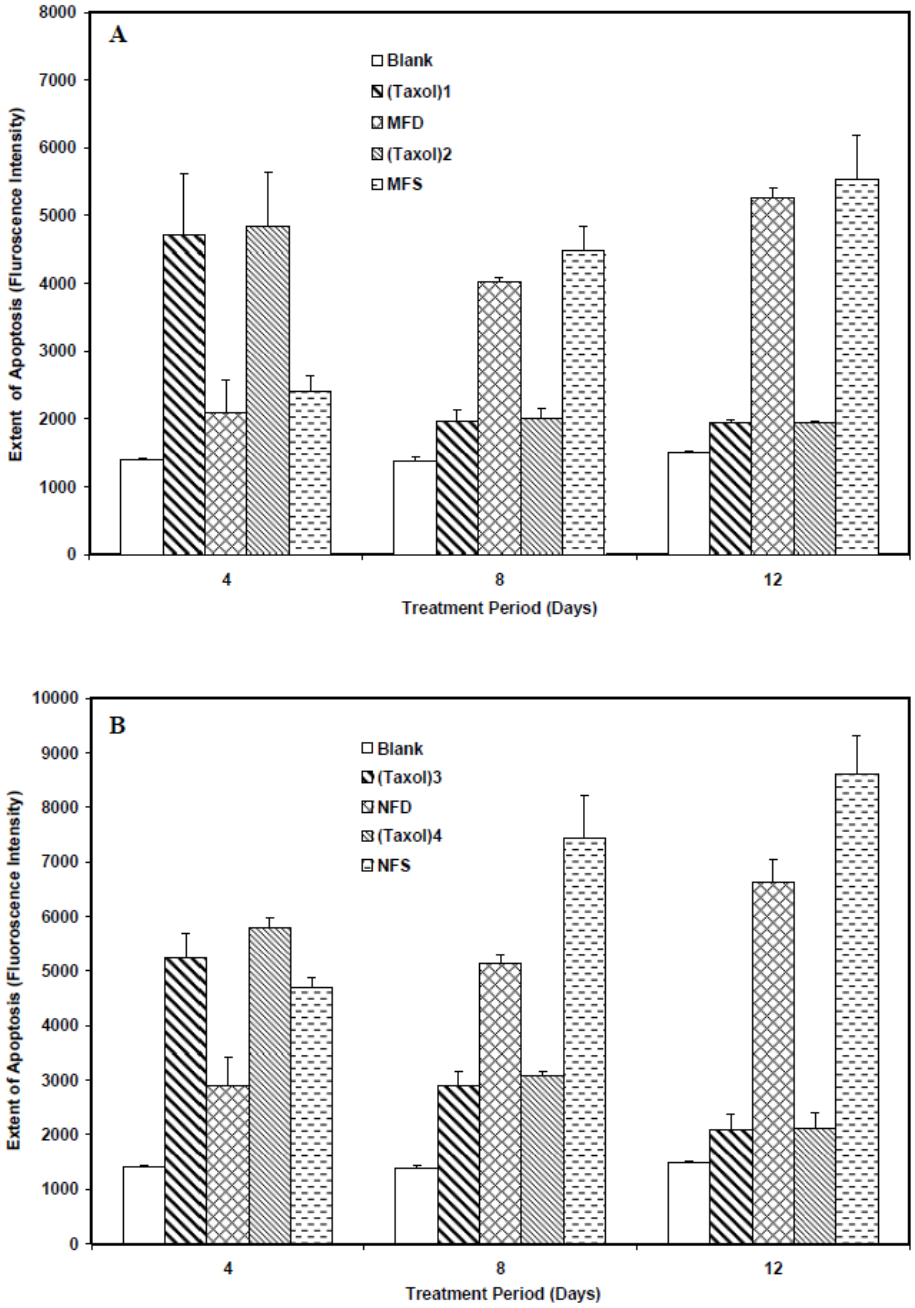

Figure 3: Extent of apoptosis in C6 glioma cells in vitro treated with different groups after 4, 8 and 12 days (a*dopted from [3])*. (A) Blank, $(Taxol)_1$, MFD, $(Taxol)_2$, MFS; (B) Blank, $(Taxol)_3$, SFD, $(Taxol)_4$, SFS. $(Taxol)_1$, $(Taxol)_2$, $(Taxol)_3$, $(Taxol)_4$ represent equivalent amounts of commercial Taxol® corresponding to paclitaxel release from MFD, SFD, MFS and SFS after 12 days (from *in vitro* release profiles). Each data point represents the average of three samples and error bars represent standard deviation.

Cellular recovery observed in Taxol® treated groups was indicative of the limitations of systemic drug administration because it only provided short and acute exposure due to low terminal half life of paclitaxel. But for the fiber treated groups, due to sustained release of paclitaxel at therapeutic concentrations, we could observe continued decrease in cell number even after 12 days (data not shown). Sustained increase in apoptotic activity for the fiber treated groups was indicative of continued paclitaxel induced apoptosis. Furthermore probably due to higher release rate from submicrofiber formulations, higher apoptotic activity was observed compared to microfiber treated group. This result also confirms GPC and *in vitro* release results.

In vivo Characterization

In vivo Subcutaneous Tumor Inhibition

This study was aimed at evaluating the efficacy of MFD, SFD, MFS and SFS in comparison with placebo fiber discs and commercial Taxol® in inhibiting subcutaneous C6 glioma tumor in BALB/c nude mice after surgical implantation. It was observed from Fig.4 that for animals with placebo discs the tumor grew unabatedly (more than 2000 mm^3) till day 24 after which the animals had to be sacrificed owing to animal ethics consideration and IACUC regulations on maximum allowable tumor size (~ 1.7 cm in diameter). Tumor volume inhibition (TVI) was calculated using the formula ($TVI = [V_C-V_E]/V_C$), where V_C and V_E represent the tumor volumes of animals from control and experimental groups respectively. Table 2 depicts TVI (%) for MFD, SFD, MFS and SFS against placebo and Taxol® control groups after 24 and 32 days.

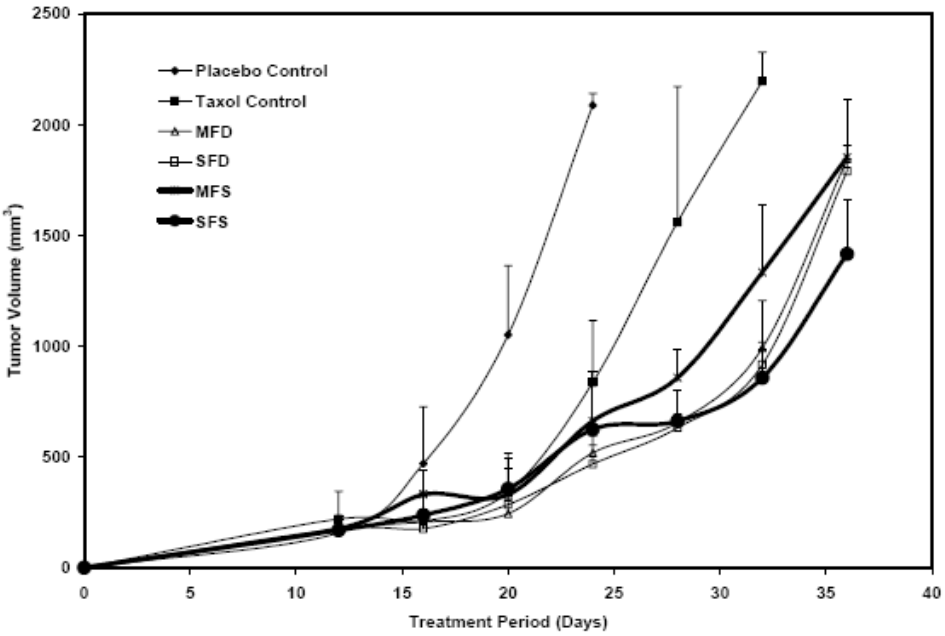

Figure 4: In vivo subcutaneous tumor inhibition profiles for different treatment groups (a*dopted from [3]*. (◆) Placebo control; (■) Taxol® control; (△) MFD; (□) SFD; (x) MFS; (●) SFS. Each data point represents five samples (n = 5 animals). Animals were sacrificed after they reached a specified tumor volume (~ 1.7 cm in diameter) irrespective of day of treatment. Error bars represent the standard deviation.

Table 2: In vivo subcutaneous tumor volume inhibition (%) for MFD, SFD, MFS, SFS against placebo and Taxol® control. (TVI: Tumor volume inhibition)

Dosage Forms	TVI (%) against Placebo control (day 24)	TVI (%) against Taxol® control (day 24)	TVI (%) against Taxol® control (day 32)
MFD	75 ± 2	38 ± 6	55 ± 6
SFD	78 ± 6	44 ± 11	59 ± 4
MFS	69 ± 9	21 ± 2	40 ± 13
SFS	71 ± 12	26 ± 2	61 ± 5

In comparison to spray dried microsphere disc having a very low paclitaxel release rate, and EHDA microspheres with a very high initial burst and release rate [1], in the present study the animals having fiber implants had approximately 45% to 60% and 40% to 55% smaller tumors respectively. This shows that a constant paclitaxel release rate within the therapeutic limit could enhance the therapeutic efficacy. The tumor volume inhibition results positively show that the fibrous dosage forms are able to provide sustainable paclitaxel release for at least more than a month *in vivo* which was reflected in the considerable tumor volume inhibition effects when compared to the control groups. Even though Taxol® group could inhibit the tumor significantly initially, it could not extend the effect for long because of its low terminal half life of less than a day *in vivo*. Once the drug cleared off, the tumor growth rate approximated that of placebo group and reached more than 2000 mm^3 after 32 days. But for the fiber treated animals tumor volume was relatively smaller (less than 1000 mm^3)

until day 32 expect for MFS (~ 1300 mm^3). However, fibers discs could be ideal for intracranial chemotherapy because of its low initial burst, sustained drug release and compactness (with higher drug loading).

Comparative study on various PLGA-paclitaxel loaded fiber discs

Fiber diameter, distribution, morphology and paclitaxel encapsulation efficiency

Six fiber disc dosage forms were fabricated using electrospinning process as described earlier and Table 3 shows the process parameters, the co-polymer used, fiber diameter and encapsulation efficiency.

Dosage Forms	Co-polymer used	Polymer concentration (% w/v)	Flow rate (ml/h)	Voltage difference between nozzle & ground (kV)	Fiber mean diameter	Paclitaxel encapsulation efficiency (%)
E1	PLGA 85:15	30	6.0	12 - 16	3.5 ± 0.32 µm	98 ± 4.9
E2	PLGA 85:15	20	0.2	16 - 18	0.92 ± 0.13 µm	93 ± 1.04
E3	PLGA 85:15	8	0.5	18 - 20	92 ±6 nm	90 ± 0.81
F1	PLGA 50:50	30	6.0	12 - 16	3.16 ± 0.3 µm	98 ± 3.0
F2	PLGA 50:50	30	0.3	16 - 18	0.93 ± 35 µm	94 ± 0.57
F3	PLGA 50:50	8	0.2	18 - 20	98 ± 23 nm	90 ± 1.09

Microfibers were fabricated at higher flow rates, polymer concentration and voltage difference compared to sub-microfiber and nanofiber. In nanofiber fabrication, to avoid formation of beads at low polymer concentrations, 20 mM of organic salt (TATPB) was used. The salt increased the conductivity of the solution, thus resulting in continuous nanofibers. Figure 5 shows that the fibers are monodisperse with smooth morphology and have good encapsulation efficiency of 90 to 98 %.

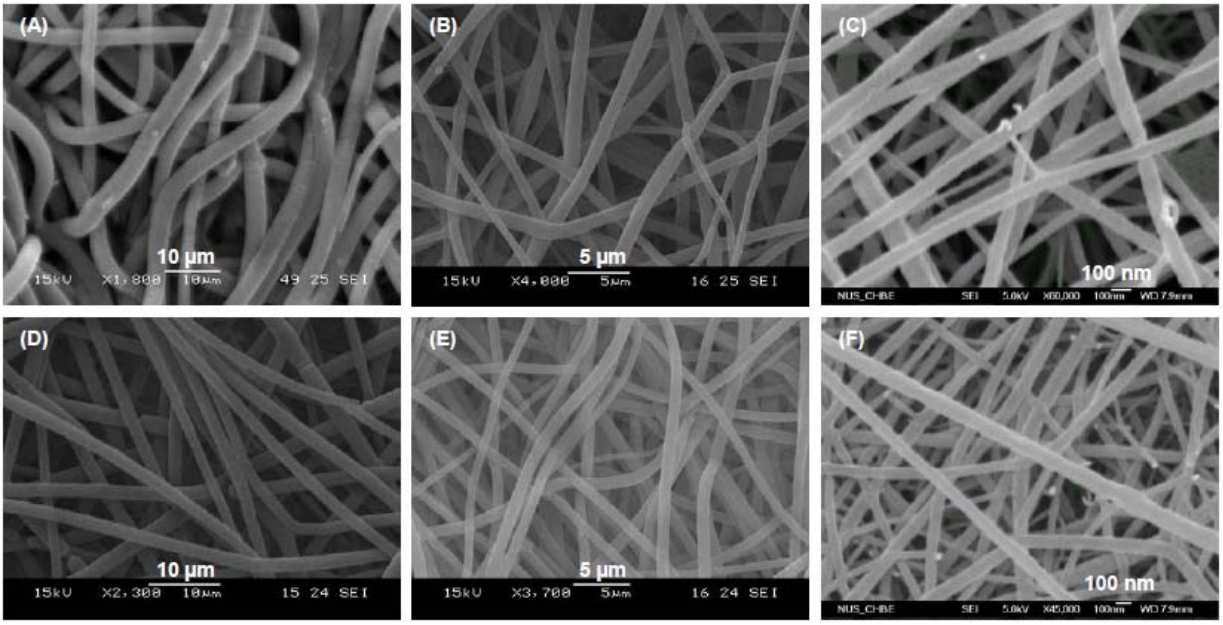

Figure 5: SEM and FESEM images of PLGA-paclitaxel electrospun fibers. (A) PLGA 85:15 microfiber disc (E1); (B) PLGA 85:15 sub-microfiber disc (E2); (C) PLGA 85:15 nanofiber disc (E3); (D) PLGA 50:50 microfiber disc (F1); (E) PLGA 50:50 sub-microfiber disc (F2); (F) PLGA 50:50 nanofiber disc (F3).

In vitro paclitaxel release profile

This study aimed at investigating the in vitro drug release profile from each of the six dosage forms and comparing them to evaluate the effect of co-polymer and fiber diameter on release profiles. Clearly, from Figure 6, we notice that the effect of co-polymer on release kinetics at the same fiber diameter is not that significantas the profiles for PLGA 85:15 and PLGA 50:50 are similar. But it is quite obvious that the fiber diameter has a very significant effect on the release rate especially when nanofibers are compared with sub-micro and microfibers. This highlights the fact that increasing the surface area to volume ratio using nanofiber would augment polymer degradation and drug release. Even PLGA 85:15 degrades at comparable rate to PLGA 50:50 when fabricated as nanofibers. At all the three scales, PLGA 50:50 exhibits slightly higher release rate compared to PLGA 85:15 owing to its greater hydrophilicity.

More importantly, unlike other available dosage forms for delivering paclitaxel [1, 5] these exhibit relatively smaller initial burst and near constant release profile. Owing to slightly higher release rate, for further in vivo studies we screened F3 and F2 as the dosage forms to be applied. F3 has fast release profile while F2 has slow release profile and hence would provide the neurosurgeons to choose between for patient specific glioma chemotherapy.

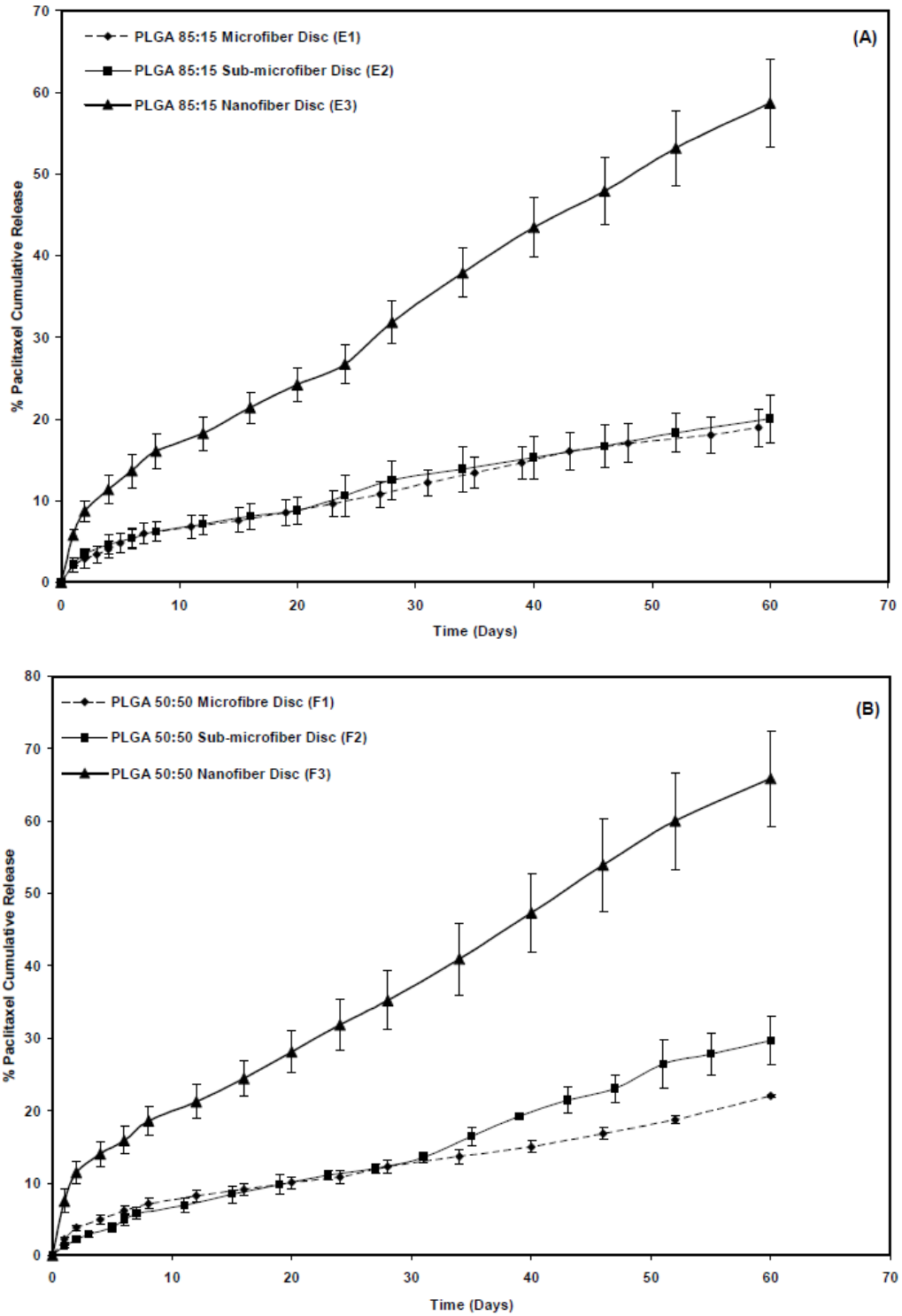

Figure 6: In vitro paclitaxel release from different dosage forms. (A) Paclitaxel loaded PLGA 85:15 fiber discs; (B) Paclitaxel loaded PLGA 50:50 fiber discs. Each data point represents the average of triplicate samples and error bars represent standard deviation.

CONCLUSIONS

The study showed that electrospinning was successfully used to fabricate monodispersed fibers with high drug encapsulation efficiency. As proof of concept, the four dosage forms (MFD, MFS, SFD, SFS) were shown to provide sustained paclitaxel release for more than 80 days with low initial burst which exhibits clear advantage over conventional systemic delivery of Taxol®. Submicrofiber discs/sheets exhibited faster release compared to microfiber discs/sheets. *In vitro* cellular apoptosis study suggested that the fiber discs/sheets were better than acute Taxol® administration against C6 glioma cells in terms of drug sustainability and cytotoxic effect. *In vivo* tumor inhibition study against subcutaneous C6 glioma in BALB/c nude mice showed that the fiber discs/sheets treated animals had much smaller tumors on day 24 and day 32 post tumor inoculation when compared to placebo control and Taxol® control groups confirming sustained release of paclitaxel and improved tumor inhibition. Hence PLGA microfiber discs/sheets could be utilized as drug delivery implants to deliver paclitaxel for post-surgical chemotherapy against malignant brain tumors.

Next, fibers discs were selected for further studies since they could be ideal for intracranial chemotherapy because of low initial burst, sustained drug release and compactness. Six fiber disc dosage forms (E1, E2, E3, F1, F2, F3) were fabricated by varying co-polymer and fiber diameter. It was shown that the co-polymer had very little effect on drug release rate compared to fiber diameter. Nanofiber discs demonstrated a significantly higher release rate in comparison to sub-micro and microfibers of the same co-polymer. But all the six dosage forms exhibited inhibited initial burst and near constant drug release rate. However, due to higher release rates, F3 and F2 were screened for further *in vivo* characterizations involving intracranial experiments such as drug biodistribution study, tumor regression study through non-invasive bio-imaging techniques and are warranted.

REFERENCES

[1] Naraharisetti PK, Ong BYS, Xie JW, Lee TKY, Wang CH, Sahinidis NV. In vivo performance of implantable biodegradable preparations delivering paclitaxel and etanidazole for the treatment of glioma. *Biomaterials* 2007;28(5):886-894.

[2] Wang J, Ng CW, Win KY, Shoemakers P, Lee TKY, Feng SS, et al. Release of paclitaxel from polylactide-co-glycolide (PLGA) microparticles and discs under irradiation. *J Microencapsulat* 2003;20(3):317-327.

[3] Ranganath SH, Wang CH. Biodegradable microfiber implants delivering paclitaxel for post-surgical chemotherapy against malignant glioma. *Biomaterials* 2008;29:2996-3003.

[4] von Eckardstein KL, Patt S, Kratzel C, Kiwit JCW, Reszka R. Local chemotherapy of F98 rat glioblastoma with paclitaxel and carboplatin embedded in liquid crystalline cubic phases. *J Neuro-oncol* 2005;72:209-215.

[5] Li KW, Dang W, Tyler BM, Troiano G, Tihan T, Brem H, et al. Polilactofate microspheres for paclitaxel delivery to central nervous system malignancies. *Clin Cancer Res* 2003;19:3441-3447.

[6] Xie J, Wang CH. Electrospun micro- and nanofibers for sustained delivery of paclitaxel to treat C6 glioma *in vitro*. *Pharma Res* 2006;23(8):1817-1826.

[7] Walter KA, Cahan MA, Gur A, et al. Interstitial Taxol delivered from a biodegradable polymer implant against experimental malignant glioma. *Cancer Res* 1994;54:2207-2212.

[8] Sampath P, Rhines LD, DiMeco F, Tyler BM, Park MC, Brem H. Interstitial docetaxel (Tarotere), carmustine and combined interstitial therapy: a novel treatment for experimental malignant glioma. *J Neurooncol* 2006;80:9-17.