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Controlled delivery of Paclitaxel from micro-porous foams for the post-surgical treatment of Glioma Blastoma Multiforme

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Introduction

Porous polymer foams have important applications in tissue engineering and potential applications in drug delivery systems. Poly $_{DL}$ lactide-co-glycolide (PLGA) is one of the most popular biodegradable polymers used in porous foam fabrication as it is biocompatible and FDA approved. Degradation rate of the foam may also be controlled by careful selection of the copolymer composition and molecular weight. By using high pressure supercritical CO_2 as a foaming agent, the use of organic solvent may be minimized or even eliminated in the production of PLGA foams as shown in previous studies. A modification of the high pressure foaming technique is presented in this study by foaming drug-loaded polymer powders. It was found that, by using polymer powders, more compact foams with smaller pores may achieved. The porous structure of the foams allows more effective drug release of the highly hydrophobic drug paclitaxel as compared to compressed discs. The foams also have good mechanical strength as required for implantation for the controlled delivery of anticancer drugs. In the present study, PLGA foams encapsulating paclitaxel for anticancer drug delivery were fabricated for the first time by a new method of foaming drug loaded PLGA powders.

A new implant formulation using the micro-porous foams in the form of discs and rods was developed for the sustained delivery of paclitaxel.

Materials and Methods

Materials

PLGA with varying lactide to glycolide ratio was used in this study. PLGA 5050 Low IV was purchased from Lakeshore Biomaterials (Birmingham, AL) and PLGA 8515 (Cat No. 430,471) was purchased from Sigma Aldrich (St Louis, MO, USA). Phosphate Buffered Saline (PBS, pH = 7.4) was purchased from Sigma Aldrich (St Louis, MO, USA). Paclitaxel was a generous gift from Bristol Myers Squibb. Compressed CO_2 (Airliquide Paris, France) was purchased from Soxal (Singapore Oxygen Air Liquide Pte Ltd). Dichloromethane (DCM) (DS1432, HPLC/Spectro Grade) and acetonitrile (ACN) (AS1122, HPLC/Spectro Grade) were purchased from Tedia (Farfield, OH, USA). Millipore water (Millipore Corporation, Billerica, MA) was used throughout the study.

Micro-porous foam fabrication using CO_2 foaming technique

The experimental setup for the fabrication of micro-porous drug-loaded foams by a CO_2 foaming technique is shown in figure 1a. Paclitaxel-loaded PLGA powders were fabricated by using a spray drying method. A custom-made mold with a height and diameter of 10 mm was machined from a ½" MNPT plug as shown in Figure 1a. For each experiment, approximately 80mg of polymer was loaded into the mold and sealed with a stainless steel wire mesh. The mold was then fitted onto the high pressure vessel and placed in a circulating water bath maintained at 35 ^{o}C. CO_2 pressure of 6 MPa was applied and an equilibration time of 60 min was used for all the foaming experiments. After saturating the polymer with CO_2, the pressure was gradually decreased from 6MPa to atmospheric pressure at a

rate of approximately 1.5MPa/min. Following the venting step, the foamed samples were removed from the mold for further analysis.

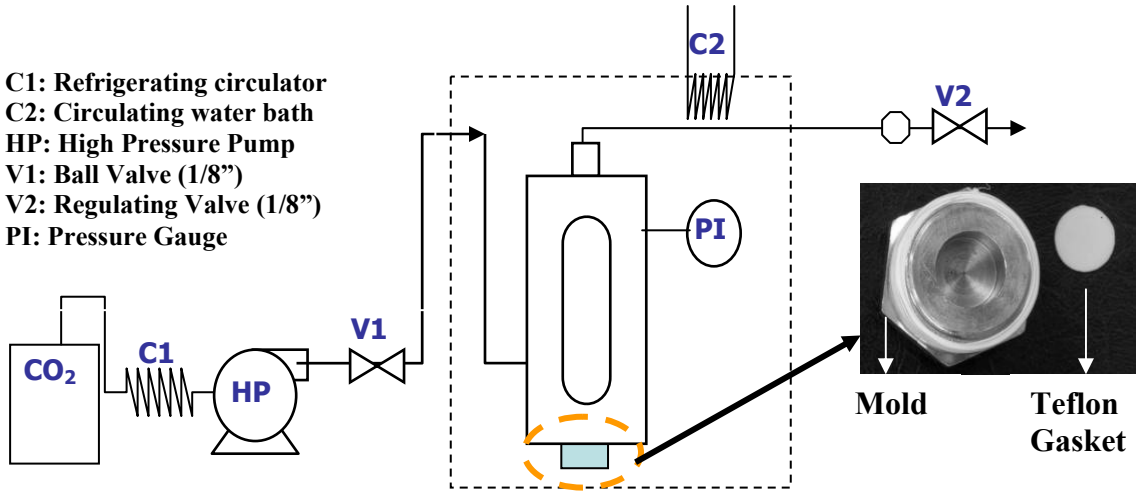

Figure 1a. Experimental setup for fabrication of micro-porous foams using high pressure CO_2 foaming method

Figure 1b (i) and (ii) shows the macroscopic state of the paclitaxel loaded PLGA before and after the foaming process respectively. Cylindrical disc of 3mm diameter x 1mm height and rods of 1mm x 1mm x 7mm were cut from the foam in (ii).

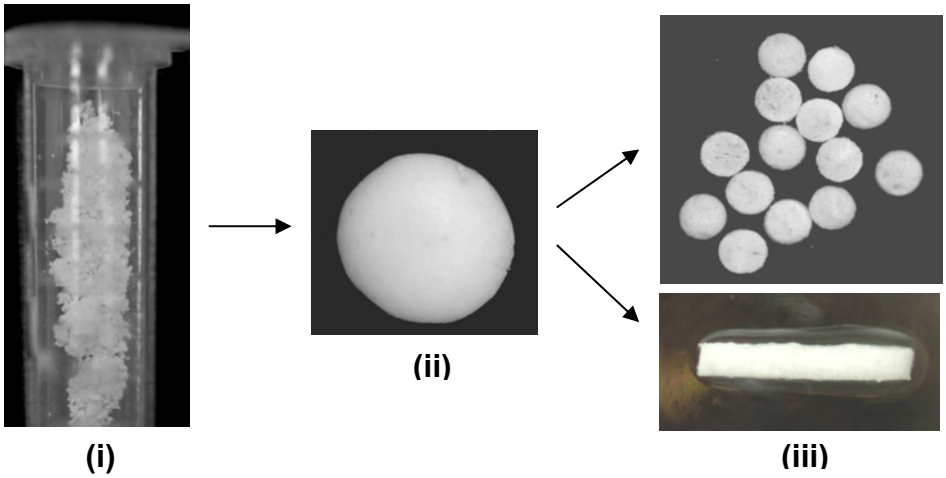

Figure 1b. Fabrication of microporous foams for controlled release;
(i) Paclitaxel-loaded powders produced using spray drying technique;
(ii) Microporous foams fabricated using the CO_2 foaming technique;
(iii) 3mm discs and 1mm x 1mm x 7mm rods obtained from (ii)

In vitro Characterization
Scanning Electron Microscopy (SEM, JEOL JSM-5600 LV, Japan) and Field Emission Scanning Electron Microscopy (FESEM, JEOL , Japan) was used to study the porous structure and surface morphology of the polymeric foams fabricated in this study.

High pressure liquid chromatography (HPLC with UV-visible detectors, Shimadzu) was used to determine the encapsulation efficiency (%) and *in vitro* release profile of the paclitaxel-loaded discs. Discs 3mm in diameter by 1mm in height were cut from the Paclitaxel loaded foams and placed in PBS solution in a shaking water bath at 37 ^{o}C and 120 rpm. At predetermined time intervals, PBS solution was removed for sampling and fresh buffer was replaced. An ACN: water (50:50 v/v) solution was used as the mobile phase for HPLC analysis.

In vivo Release Profile
The release profile of the foams within an in vivo environment was evaluated through surgical implantation of the foams into the subcutaneous flank of BALB/c mice. At regular intervals of one week, one experimental group (n = 5) was sacrificed. The foam was extracted from the flanks of the animals and analyzed by HPLC for remaining encapsulated drug in the foams.

Weight of the animals was also closely monitored for signs of systemic toxicity from failure of the foams. Experimental results will be presented during the AIChE meeting.

In vivo Penetration Analysis
In order to evaluate the penetration of paclitaxel from the foams into a brain environment, foam rods encapsulating the drug were implanted into the brains of Wistar Rats up to stipulated end points of 14, 21 and 28 days at which the respective experimental groups were sacrificed. The brains were harvested and sectioned coronally in 1 mm thick slices from the site of implantation and each slice was weighed, homogenized and analyzed by LcMSMS for the amount of paclitaxel present. Experimental results will be presented during the AIChE meeting.

Results and Discussion
In vitro studies
The physical properties and controlled release performance of 2 types of PLGA foams were evaluated in this study. Figures 2a and 2b illustrate the porous matrix structure of the PLGA 5050 and PLGA 8515 foams respectively. It can be observed from figure 2a that PLGA 5050 foam has more uniform pore size distribution with interconnecting pores. On the other hand, in Figure 2b, PLGA 8515 foam has a larger pore size distribution and closed pores. This may be explained by the property of the polymer material used. PLGA 5050 is a more amorphous polymer than PLGA 8515 due to the lower lactide content in its

copolymer chain. Therefore the walls between interconnecting pores will stretch more thinly during the venting step and the walls eventually rupture and tear, leaving an open pore structure shown in Figure 2a.

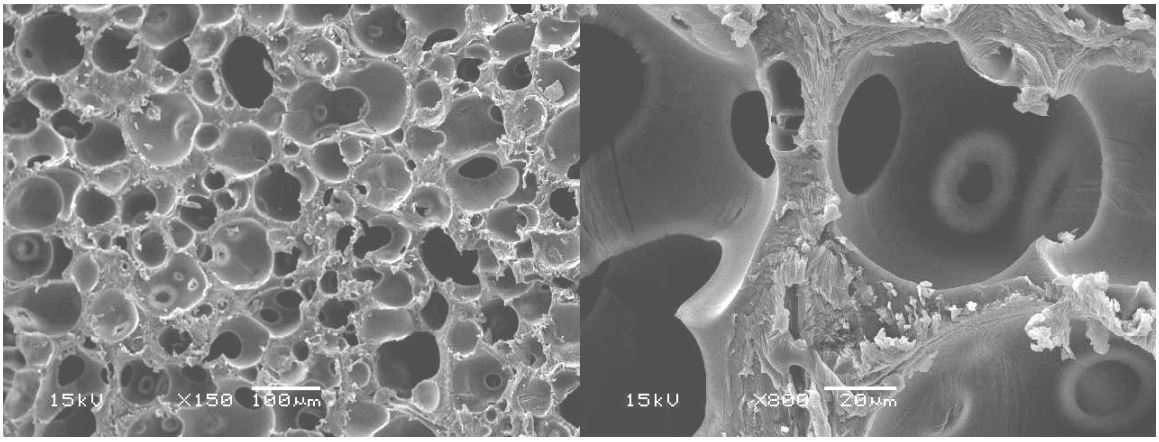

Figure 2a. Scanning Electron Micrographs of the porous structure of 5% paclitaxel loaded PLGA 5050 foams

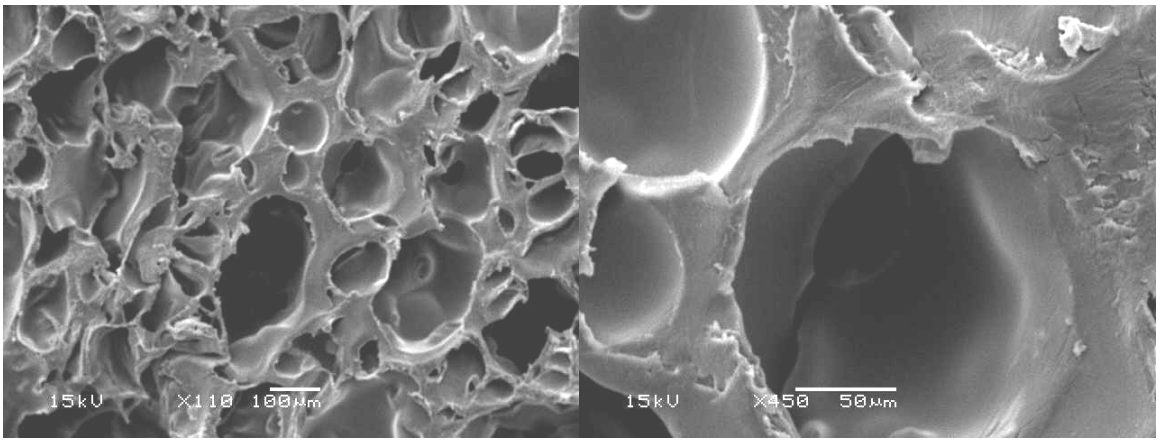

Figure 2b. Scanning Electron Micrographs of the porous structure of 5% paclitaxel loaded PLGA 8515 foams

The encapsulation efficiency of the foaming process was determined as:

$$Encapsulation\ efficiency\ (\%) = \frac{drug\ loading\ in\ powders\left(mg/mg\right)}{drug\ loading\ in\ foam\left(mg/mg\right)} \qquad (1)$$

Using HPLC analysis for the determination of the actual drug loading in both the spray dried powders and foam, the encapsulation efficiency of paclitaxel during the foaming process was found to be 99.4% (close to 100%).

The *in vitro* release profiles of paclitaxel from a PLGA 5050 disc obtained by compression-molding [1], PLGA 5050 Microspheres [2], and PLGA 5050 foams are shown in Figure 3. The release of paclitaxel from compressed discs is found to be very slow with less than 3% release after one month. Drug release from the microporous foams is shown to be much faster than from the compressed disc. This may be attributed to the highly porous structure of the foam matrix with a much higher surface area for faster drug release.

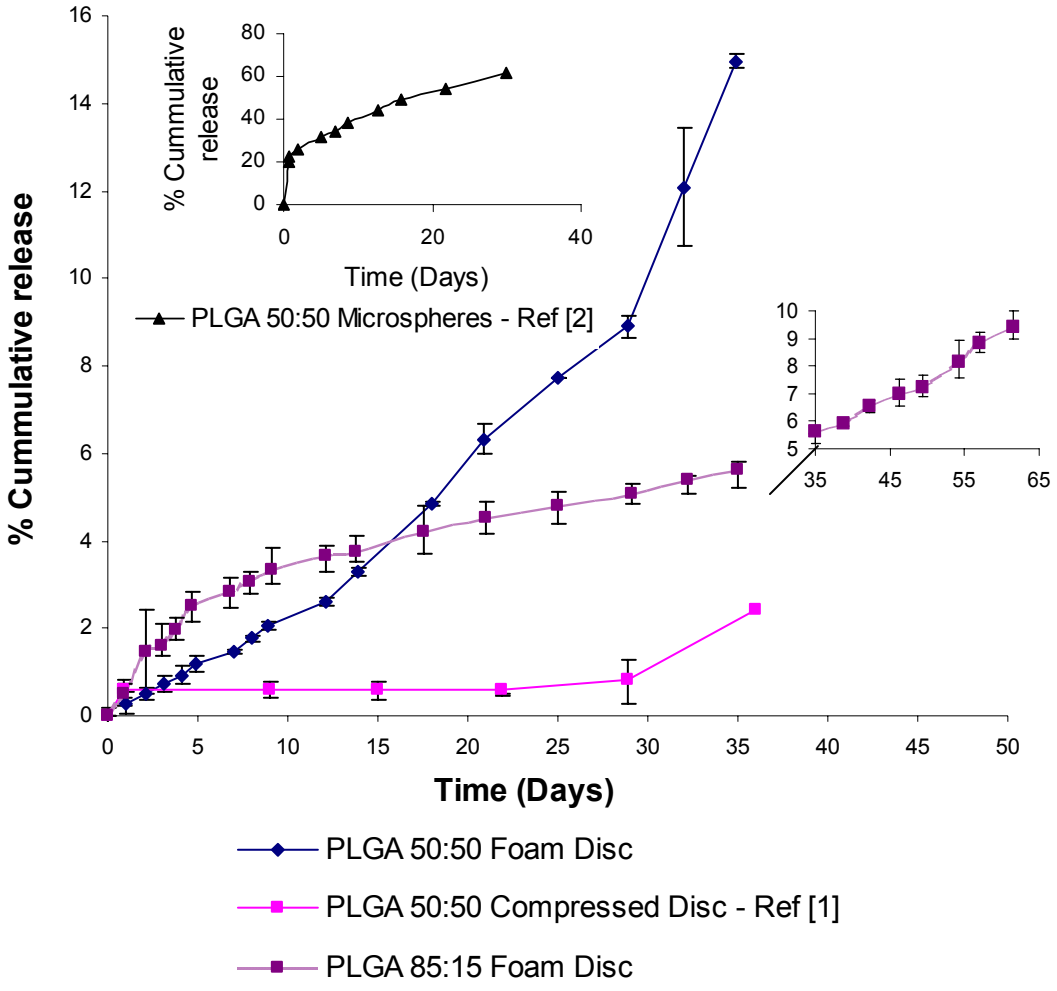

Figure 3. *In vitro* release profile of paclitaxel from PLGA 5050 microspheres and compression-molding discs, PLGA 5050 foams and PLGA 8515 foams

The *in vitro* release profile of paclitaxel from PLGA 8515 foams is shown in Figure 3. After 15 days, PLGA 5050 gives a faster release rate as compared to PLGA 8515. At the end of 35 days, approximately 15% of the drug has been released for PLGA 5050 foams. For PLGA 8515 foams, the release after 60 days is less than 10%. These observations are consistent with the foam matrix structure, as PLGA 5050 has an open pore structure and PLGA 8515 has a closed pore structure. PLGA 5050 has a higher glycolide content which makes it

more susceptible to hydrophilic attack by the aqueous medium during *in vitro* release studies. The lower molecular weight of the PLGA 5050 polymer used in this study also contributed to the higher degradation of the polymer which leads to a higher drug release rate.

Conclusions

Paclitaxel-loaded PLGA foams were fabricated for controlled delivery applications. These foams provide an alternative to conventional methods of making implants using compression discs. Micro-porous foams have higher surface-to-volume ratios, allowing more efficient drug release. At the same time, the foams are mechanically strong enough to be used as an implant in surgical experiments. By altering the co-polymer ratio of the PLGA used, the release rate of paclitaxel may be modified to address therapeutic requirements.

Further experimental results for *in vivo* release profiles and penetration to surrounding brain tissue for the paclitaxel-loaded PLGA foams will be given at the meeting.

Acknowledgements

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